UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

Dottorato di Ricerca in
Scienze e Tecnologie delle Produzioni Agro-Alimentari
XXVI Ciclo

PhD Thesis

INNOVATIVE APPROACHES FOR EVALUATION OF DIETARY EXPOSURE TO TOXIC PERSISTENT AND EMERGING CONTAMINANTS.

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ABSTRACT

Persistent, Bioaccumulative and Toxic (PBT) Contaminants are toxic substances mainly released into the environment as a result of human activities (eg, industrial production, agro-animal husbandry practices, waste production/disposal). They are equipped with a high intrinsic stability that allows them to remain unchanged in the environment for a long time, undergoing a wide geographical spread between environmental compartments (air, water, soil and biota) and accumulating in living organisms to harmful levels for both human health and ecosystem (www.epa.gov/pbt).

Population exposure to these PBT contaminants mainly takes place through food (Pazefall, 2002), therefore their monitoring is fundamental for Public Health.

The research described in this doctoral thesis mainly concerned the identification of some of these contaminants in meals, as well as actually consumed by the population. The main objective of the research activity was the development of a method to determine the real contribution of the daily diet to the toxic and persistent contaminants exposure for people living in different geographical areas of Italy.

In particular, the interest has been directed to the categories of substances in the families of polychlorinated biphenyls (PCBs), dioxins and furans (PCDDs, PCDFs) and polybrominated diphenyl ethers (PBDEs).

Our attention was focused on Total diet studies (TDS) and Duplicate diet studies (DDS), through the analysis of canteens and restaurants meals from Italian adults and children and the analysis of double portions of all meals (24h) prepared at home by some adults respectively.

Sampling was carried out through the selection of six different types of meals, depending on the following different dietary habits: consumption of baby food by toddlers aged 9-12 months (Baby food, BF); consumption of meals in school canteens for children attending nursery and primary school (age 4-9 years), and canteens for adults (aged 18-64 years); consumption of fast food meals by adults (Fast Foods, FF); consumption of food prepared at home by adults (Duplicate diet, DD); consumption of meals by adults that observe a vegetarian lifestyle (Vegetarian diet, V); consumption of "Sundays meals " at the restaurant by adults (Restaurant meals, RM). In particular, canteen meals were collected in collaboration with four Italian primary schools (Ferrara, Perugia, Genoa-1, Genoa-2), a nursery school (Portici, NA) and an office canteen (Brescia). All the composites were analyzed by gas chromatography coupled to mass spectrometry (HRGC-HRMS for PCDD/F and PCB and by HRGC-LRMS for PBDE).

Analysis of the obtained data revealed no exposure beyond the legal limits for the examined population, the estimates were lower even than those of the rest of the European population. Only for some restaurant meals RM higher values compared to the general trend were obtained, probably caused by the additional presence, in the above menu, of meat based dressing re-fill in the first courses (i.e.tagliatelle alla bolognese and agnolotti) and of dessert prepared with milk and dairy products. Foods of animal origin, in fact, generally show higher levels of bioaccumulative pollutants compared to foods of plant origin (EFSA 2011a, 2012b EFSA). This explanation is supported by the lower levels of contaminants found in the vegetarian meal (V).

For all three categories of PBT contaminants (dioxins, furans, PCBs and PBDEs) a higher value was obtained babies and children meals, in line with the general view which explains that they should be more exposed than the adults because of greater consumption of food relative to their body weight (EFSA 2012A).

Due to the complexity and expensiveness of conducting long-term studies, such as TDS, the analysis of composites as result of pooled meals may represent a first cost-effective screening useful to assess persistent organic pollutants intake through food as actually consumed.

In order to refine the dietary exposure assessment it may be useful to perform such screening on regular basis thus reducing possible uncertainties related to the meals representativeness also on seasonal basis.

RIASSUNTO

I contaminanti Persistenti, Bioaccumulabili e Tossici (PBTC) sono sostanze tossiche rilasciate nell'ambiente, soprattutto in conseguenza di attività antropiche (es., produzione industriale, pratiche agro-zootecniche, produzione/smaltimento di rifiuti). Sono dotati di una elevata stabilità intrinseca che permette loro di rimanere inalterati nell'ambiente a lungo, subire un'ampia diffusione geografica tra comparti ambientali (aria, acqua, suolo e biota) e accumulare negli organismi viventi fino a livelli nocivi per la salute umana e per l'ecosistema (www.epa.gov/pbt).

L'esposizione a tali contaminanti nella popolazione avviene principalmente attraverso gli alimenti (Pazefall 2002), pertanto il loro monitoraggio risulta di particolare rilevanza per la Sanità Pubblica.

L'attività di ricerca oggetto del presente lavoro di tesi di dottorato ha riguardato essenzialmente l'identificazione di alcuni di questi contaminanti in pasti completi, così come effettivamente consumati da cittadini italiani che vivono in diverse aree geografiche del nostro paese.

In particolare l'interesse è stato rivolto alle categorie di sostanze che rientrano nelle famiglie dei policlorodibenzodiossine e policlorodibenzofurani (PCDD, PCDF), policlorobifenili (PCB) e dei polibromodifenileteri (PBDE).

La nostra attenzione si è concentrata su studi di *Total diet* (TDS) e studi di *Duplicate diet* (DDS) rispettivamente attraverso l'analisi dei pasti consumati nelle mense e nei ristoranti italiani da adulti e bambini e l'analisi delle porzioni doppie di tutti i pasti (24h) preparati a casa da alcuni adulti.

Il campionamento è stato effettuato attraverso la selezione di sei differenti tipologie di pasti, in funzione delle seguenti abitudini alimentari: consumo di alimenti per l'infanzia da bambini di età compresa tra 9-12 mesi (*Baby food*, BF); consumo di pasti presso mense scolastiche, per bambini che frequentano la scuola materna ed elementare (età 4-9 anni), e mense aziendali per adulti (di età compresa tra 18-64 anni); consumo di pasti presso fast food da parte di adulti (*Fast food*, FF); consumo di cibo preparato a casa da adulti (*Duplicate diet*, DD); consumo di pasti da adulti che osservano uno stile di vita vegetariano (*Vegetarian diet*, V); consumo dei "pasti della domenica" al ristorante da parte di adulti (*Restaurant meals*, RM). In particolare, i pasti delle mense sono stati raccolti in collaborazione con quattro scuole elementari italiane (Ferrara, Perugia, Genova-1, Genova-2), una scuola materna (Portici, NA) e una mensa che fornisce pasti per ufficio (Brescia). Tutti i campioni sono stati analizzati mediante gas cromatografia accoppiata alla spettrometria di massa (HRGC-HRMS per PCDD/F e PCB e HRGC-LRMS per PBDE).

Dall'analisi dei dati ottenuti non è emersa alcuna esposizione oltre i limiti di legge per la popolazione presa in esame, le stime sono risultate inferiori anche rispetto a quelle relative al resto della popolazione europea.

Soltanto per alcuni "pasti della domenica" (RM-D e RM-E) sono stati ottenuti valori più alti rispetto al *trend* generale, probabilmente determinati dalla presenza, nel menù, di primi piatti a base di carne (ad es. tagliatelle al ragù o agnolotti). Gli alimenti di origine animale, infatti, mostrano generalmente livelli più alti di inquinanti bioaccumulabili rispetto agli alimenti di origine vegetale (EFSA 2011a, 2012b EFSA). Tale spiegazione è supportata dai più bassi livelli di inquinanti riscontrati nel pasto vegetariano (V).

Per tutte le tre categorie di PBTC in esame (diossine, furani, PCB e PBDE) è stato ottenuto un valore più elevato nei pasti per neonati e bambini, in linea con la considerazione generale secondo la quale i più piccoli dovrebbero essere più esposti rispetto agli adulti a causa del maggiore consumo di cibo relativamente al loro peso corporeo (EFSA 2012A).

L'analisi di compositi come risultato di pasti aggregati può rappresentare uno screening iniziale conveniente e utile per la valutazione dell'esposizione di inquinanti organici persistenti attraverso il cibo, nella forma effettivamente consumata.

Al fine di perfezionare la valutazione dell'esposizione alimentare, potrebbe essere utile eseguire tale screening su base regolare, riducendo così eventuali incertezze legate alla rappresentatività dei pasti anche su base stagionale.

1. STATE OF THE ART

Persistent, Bioaccumulative and Toxic Contaminants (PBTC) are toxic substances mainly released into the environment as a result of human activities (eg, industrial production, agro-animal husbandry practices, waste production/disposal). They are equipped with a high intrinsic stability that allows them to remain unchanged in the environment for a long time, undergoing a wide geographical spread between environmental compartments (air, water, soil and biota) and accumulating in living organisms to harmful levels for both human health and ecosystem (www.epa.gov/pbt).

The EPA (U.S. Environmental Protection Agency) compiled a list of these compounds (PBT Annual Report, 2000), which includes for example:

- Chlorinated pesticides such as aldrin, dieldrin, chlordane, DDT and its metabolites, hexachlorobenzene (HCB)

- Persistent organic pollutants (POPs) such as PCBs (polychlorinated biphenyls), dioxins and furans (PCDDs, PCDFs) and polybrominated diphenyl ethers (PBDEs)

- Heavy metals and their derivatives.

Population exposure to these PBT contaminants mainly takes place through food (Pazefall, 2002), therefore their monitoring is fundamental for Public Health. The research described in this doctoral thesis principally concerned the identification of some of these contaminants in food matrices and the development of innovative methods for the dietary exposure determination. In particular, the interest has been directed to the categories of substances in the families of dioxins, PCBs and PBDEs .

2. DIOXINS

2.1. General Data

The generic word "dioxin" refers to a group of 210 polychlorinated aromatic compounds, divided into two families: polychlorinated dibenzo-*p*-dioxins (PCDDs or "dioxins") and polychlorinated dibenzofurans (PCDFs or "furans"). They are toxic substances that are subject to bioaccumulation and biomagnification processes thanks to their chemical stability and the lipophilic character. Therefore, also a parts per billion environmental contamination could be dangerous for humans, the last link of the food chain.

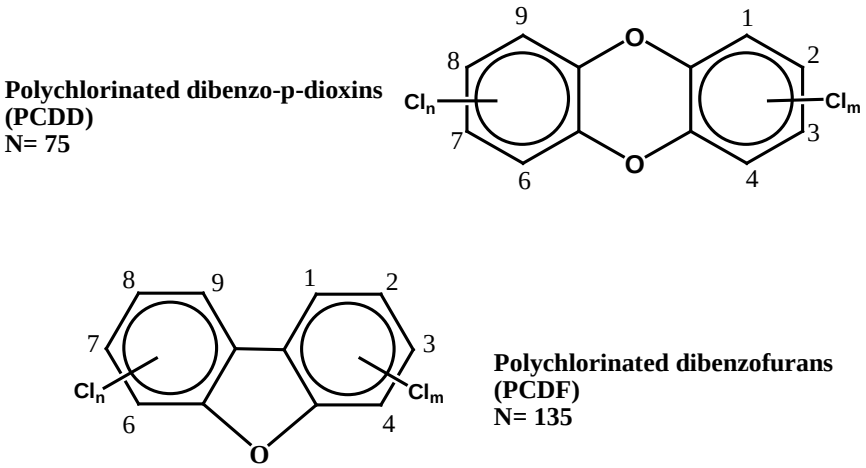

Figure 2.1: Structural formula of dioxins and furans.

There are 75 congeners of dioxins and 135 of furans (Figure 2.1), however, only 17 chlorine substituted congeners (7 PCDDs and 10 PCDFs, respectively), at the same time in at least the 2,3,7,8 positions, cause toxicological problems. The toxicity of dioxins depends on the number and position of the chlorine atoms of the aromatic ring. The most toxic congener, the 2,3,7,8-tetrachlorodibenzodioxin (TCDD), has 4 chlorine atoms linked to the β carbon atoms of the aromatic ring and no chlorine atom bound in position α. It is classified as toxicity group 1 by the International Agency for Research on Cancer IARC (Figure 2.2).

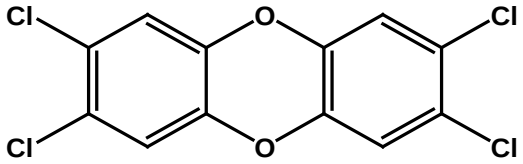

Figure 2.2: Structural formula of 2,3,7,8-TCDD.

Dioxins are thermostable substances, slightly polar, insoluble in water, highly liposoluble, highly resistant to chemical and biological degradation. They bind to the organic fraction present in the soil and, once adsorbed, remain relatively motionless: because of their insolubility in water they do not tend to migrate in depth.

2.2. Synthesis

Dioxins are never deliberately produced but are formed as unwanted byproducts of some chemical processes and/or combustion. Their formation is favored in the presence of chlorinated aromatic compounds and processes that occur at high temperatures (800-900°C). Among chemical processes, those for the production of plastics, chemicals (pesticides and chlorinated herbicides), bleaching of paper, and, in refineries, production of fuel oils emerge.

Possible dioxin sources are also combustion processes that can be distinguished in:

- uncontrolled combustion, including:
  - - Accidental fires and outdoor heterogeneous materials, such as urban waste, tires, etc.;
  - - Forest fires in the presence of chlorinated compounds for the combustion of lignin and cellulose;
  - - Volcanic eruptions with similar mechanism of production of dioxins forest fires.

• controlled combustion (voluntary) to:
    - Municipal solid waste (incineration);
    - Sludge (incineration);
    - Fuel/fuel in the process of cement production;
    - Fuel/fuel in the ferrous and non melting processes;
• other controlled combustions for energy production:
    - - Transport (for the use of fuels containing chlorinated compounds);
    - - Combustion of treated wood;
    - - Combustion of fuel oils.

Dioxin precursors are compounds as the pentachlorophenol, PCBs, chlorinated paraffins in the oils, chlorine and inorganic thermoplastics. These chemical compounds are used for the production of wood preservatives, pesticides, leather and skin in general and in the plastics industry.

2.3. Toxicity

There are two degrees of toxicity for dioxins:

-Acute Toxicity: in humans causes very persistent skin lesions (chloracne). There is also an emato-toxic action by inducing alterations in the EME biosynthetic chain.

-Chronic Toxicity: alterations in liver functions, disorders of lipid and glucose metabolism, impaired lung function, both peripheral and central neurological damage have been described.

Generally, dioxins are not detected as individual compounds, but as complex mixtures of different congeners; each congener has a different toxicity. They act primarily through interaction with the intracellular aryl hydrocarbon receptor (AhR) (Bradfield, 1991). This receptor is a member of the family of bHLH/PAS (basic Helix-Loop-Helix/Per-Arnt-Sim) proteins, known transcriptional regulators, involved in detoxification of xenobiotics, and in particular, in the various dioxin induced tissue-specific physiological responses.

The action mechanism of dioxin-AhR interaction has been widely debated and only recently defined. The AhR receptor, as a result of the bond with dioxin, enters the nucleus and forms a heterodimer with a structurally similar protein, known as AhR nuclear translocator (ARNT). The Complex AhR/ARNT, joining his free time at the ER receptor and at the p300 coactivator, recognizes a specific sequence of DNA known as HRE (Hormone Response Elements), corresponding to the enhancer/promoter region for a hormone-dependent gene.

The interaction of the DNA-receptor complex causes alterations in chromatin structure, resulting in induction of the transactivation signal. In this way, access to the TATAbox sequence of the promoter is facilitated for the transcription initiation.

One of the known targets is the CYP1A1 gene that regulates the synthesis of microsomal cytochrome P-450 (a gene system aimed to the biotransformation and elimination of toxic environmental components). The available data seem to highlight the many activities of TCDD-AHR complex, on the cell division and differentiation mechanisms, on the interactions with growth factors, on the metabolism of certain hormones.

The combination of these actions leads to the occurrence of very different consequences depending on the type of cell exposed to the action of toxic. The type of genes transcribed depends on the type of dioxin mixture, on the affinity of these to join the AhR receptor and on the concentration of the pollutant, the method of exposure and cell types more involved, and on the biological variability of the individual metabolic response. TCDD in humans is perhaps the most potent known tumor promoter, that is not acting directly as a carcinogen, but promotes tumor progression once the cell has been in contact with subcancerogene doses of a true carcinogen. Carcinogenesis by TCDD is a complex process, which varies depending on the dose, target organ, mixture of dioxins, the way of administration (Wormke M. et al, 2000; M. Warner et al 2002). To express the toxicity of individual congeners, the concept of toxic equivalency factor (TEF) was introduced. The toxic equivalency factors are based on the consideration that the PCDD/F are structurally similar compounds that have the same structural action mechanism (activation of the Ah receptor) and produce similar toxic effects. TEF are calculated

by comparing the binding affinity of the various organochlorine compounds with the Ah receptor, compared to that of 2,3,7,8- TCDD, whereas the affinity of this molecule as the reference unit value .

To express the total concentration of dioxins the concept of equivalent toxicity (TEQ) was introduced. It is obtained by summing the products between the TEF values of individual congeners and their respective concentrations:

$$TEQ = \sum_{i=1}^{n} (C_i * TEF_i)$$

Two schemes of classification have been proposed for TEF. The first was developed by NATO, and mainly used to measure the concentration levels of dioxins in different environmental matrices (water, air, soil) in relation to the quality standards set by the rules and regulations (I-TE system, International Toxicity Equivalent). The second, developed by the World Health Organization (WHO), is used to assess the degree of toxicity of these compounds, in relation to human health effects (WHO-TE system) (Table 1):

Table 2.1: NATO and WHO TEF.

PCDD/F	I-TEFs (NATO)	WHO-TEFs (1998)	WHO-TEFs (2005)
2,3,7,8-TCDD	1	1	1
1,2,3,7,8-PeCDD	0,5	1	1
1,2,3,4,7,8-HxCDD	0,1	0,1	0,1
1,2,3,6,7,8-HxCDD	0,1	0,1	0,1
1,2,3,7,8,9-HxCDD	0,1	0,1	0,1
1,2,3,4,6,7,8-HpCDD	0,01	0,01	0,01
OCDD	0,001	0,0001	0,0003
2,3,7,8-TCDF	0,1	0,1	0,1
1,2,3,7,8-PeCDF	0,05	0,05	0,03
2,3,4,7,8-PeCDF	0,5	0,5	0,3
1,2,3,4,7,8-HxCDF	0,1	0,1	0,1
1,2,3,6,7,8-HxCDF	0,1	0,1	0,1
1,2,3,7,8,9-HxCDF	0,1	0,1	0,1
2,3,4,6,7,8-HxCDF	0,1	0,1	0,1
1,2,3,4,6,7,8-HpCDF	0,01	0,01	0,01
1,2,3,4,7,8,9-HpCDF	0,01	0,01	0,01
OCDF	0,001	0,0001	0,0003

(T=tetra, Pe=penta, Hx=hexa, Hp=hepta, O=octa)

Toxic equivalency factors are periodically updated based on the new information emerging from the toxicological studies of these substances. In 2005, the scale factors of the WHO-TE has been updated, changing the previous TEF values for some congeners (Table 2.1). With Regulation (EU) No 277/2012 of 28 March 2012, Annexes I and II of 2002/32/EC, Council and European Parliament Directive were amended as regards the maximum levels and action thresholds for dioxins and polychlorinated biphenyls. The maximum levels of PCDD/F and dioxin-like PCBs (Table 2.2) have been established taking into account the most recent occurrence data. These data are collected in the "Results of the monitoring of non-dioxin-like PCBs in food and feed" EFSA scientific report.

Table 2.2 Levels of PCDD/F and dioxin-like PCBs in food and feed

Congenere	TEF	Congenere	TEF
PCDF/PCDF		**DIOXIN-LIKE PCBS**	
2,3,7,8-TCDD	1	**non-ortho PCBs**	
1,2,3,7,8-PeCDD	1	PCB 77	0.0001
1,2,3,4,7,8-HxCDD	0.1	PCB 81	0.0003
1,2,3,6,7,8-HxCDD	0.1	PCB 126	0.1
1,2,3,7,8,9-HxCDD	0.1	PCB 169	0.003
1,2,3,4,6,7,8-HpCDD	0.01		
OCDD	0.003	**mono-ortho PCBs**	
2,3,7,8-TCDF	0.1	PCB 105	0,00003
1,2,3,7,8-PeCDF	0.03	PCB 114	0,00003
2,3,4,7,8-PeCDF	0.3	PCB 118	0,00003
1,2,3,4,7,8-HxCDF	0.1	PCB 123	0,00003
1,2,3,6,7,8-HxCDF	0.1	PCB 156	0,00003
1,2,3,7,8,9-HxCDF	0.1	PCB 157	0,00003
2,3,4,6,7,8-HxCDF	0.1	PCB 167	0,00003
1,2,3,4,6,7,8-HpCDF	0.01	PCB 189	0,00003
1,2,3,4,7,8,9-HpCDF	0.01		
OCDF	0.0003		

Abbreviations: “T”=tetra; “Pe”=penta; “Hx”=hexa; “Hp”=hepta; “O”=octa; “CDD” = chlorodibenzodioxin; “CDF”= chlorodibenzofuran; “CB”= chlorobifenile.

Diet is the dioxin main source of input in the human body. The introduction of these substances through the diet for European population presents a wide variability due to different eating habits and types of supply.

Dioxins tend to mainly focus on fatty fish, dairy and beef cattle, chickens, pigs. At constant exposure, longer is the life of the animal, higher is the accumulation of dioxin in adipose tissue. Exposure to small doses also occurs through breathing and skin but diet is the main source of accumulation in the body.

3. POLYCHLORINATED BIPHENYL (PCB)

Polychlorinated biphenyls (PCBs) are synthetic persistent organic pollutants (POPs) which pose such significant threats to health and environment that on 22 May 2001, the world's governments adopted an international treaty aimed at restricting and ultimately eliminating their production, use, release and storage.
PCBs were synthesized in the United States in the late twenties by Monsanto, were patented and produced on a large scale, achieving a great commercial interest towards the beginning of the fifties. Only in the United States were produced 670,000 tons and similar amounts could be estimated also for Europe.
Their remarkable inertness toward other compounds and high heat resistance have been exploited in several industrial applications, classified into three categories (Pavan et al., 2003):

- controllable closed systems, where PCBs are used as dielectric fluids in transformers and accumulators (77% utilization) with dispersions in to the environment;
- not controllable closed systems, where PCBs were used as fluids in hydraulic systems and radiators with frequent with dispersions in to the environment;
- dissipative uses: industrial lubricants and cutting oils, additives of pesticides, copier paper, adhesives, plastic compounds and varnishes; uses that allow the direct contact of the PCBs with the environment.

In 1972 the use of PCBs in open systems, ie paints, sealing masses, textiles and paper was banned. In 1996 (Directive 96/59/EC) the general prohibition of the use of PCBs become law. Since their danger has been understood only in recent times, PCBs have been disposed of for many years, without any precaution to counteract the environment diffusion. PCBs in fact, are now widespread to every part of the globe: large quantities of PCBs have been introduced into the environment by burning or incineration, by evaporation from paints and plastics, direct discharge into sewers and waterways, eliminating uncontrolled landfill in shape, without a complete destruction of the material (ATDSR, 1993). It should be stressed that the major source of human contamination by PCBs is the dietary intake, although, in some cases, inhalation and cutaneous exposures are not to be underestimated.

3.1 Structure

Polychlorinated biphenyls (PCBs) are a class of 209 compounds, having from 1 to 10 chlorine atoms as substituents on the biphenyl molecule (Fig. 3.1).

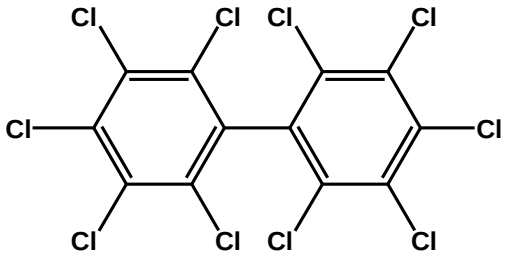

Figure 3.1 Decachlorobiphenyl

The PCBs general structure, the isomeric class distinction (congeners with the same number of chlorine atoms in the molecule), the composition of the most common commercial mixtures and the list of 209 congeners with the systematic numbering established by Ballschmiter et al are shown in Table 3.1 (Pavan et al., 2003).

Table 3.1 PCB general data

Chlorine positions on each ring	None	2	3	4	23	24	25	26	34	35	234	235	236	245	246	345	2345	2346	2356	23456
23456																				209
2356																			202	208
2346																		197	201	207
2345																	194	196	199	206
345																169	189	191	193	205
246															155	168	182	184	188	204
245														153	154	167	180	183	187	203
236													136	149	150	164	174	176	179	200
235												133	135	146	148	162	172	175	178	198
234											128	130	132	138	140	157	170	171	177	195
35										80	107	111	113	120	121	127	159	161	165	192
34									77	79	105	109	110	118	119	126	156	158	163	190
26								54	71	73	89	94	96	102	104	125	143	145	152	186
25							52	53	70	72	87	92	95	101	103	124	141	144	151	185
24						47	49	51	66	68	85	90	91	99	100	123	137	139	147	181
23					40	42	44	46	56	58	82	83	84	97	98	122	129	131	134	173
4				15	22	28	31	32	37	39	60	63	64	74	75	81	114	115	117	166
3			11	13	20	25	26	27	35	36	55	57	59	67	69	78	106	108	112	160
2		4	6	8	16	17	18	19	33	34	41	43	45	48	50	76	86	88	93	142
None	0	1	2	3	5	7	9	10	12	14	21	23	24	29	30	38	61	62	65	116

[a] Example (illustrated by shaded area in table): To determine IUPAC and alternative names for PCB 156:
(1) Locate PCB 156 within table.
(2) Identify the associated column heading (2345) and row heading (34) values.
(3) The IUPAC name for PCB 156 is 2,3,3',4,4',5-hexachlorobiphenyl.
Various additional names for this congener include 2,3,4,5,3',4'-hexachlorobiphenyl, 2345-3'4'-hexachlorobiphenyl (group starting with lower number appears first), 2345-34-hexachlorobiphenyl, and 233'44'5-hexachlorobiphenyl.
Adapted from Frame et al. (1996).

Generally, the term PCBs is related to a mixture of compounds from monochlorobiphenyls to decachlorobiphenyls, grouped in 10 classes of homologues according to their chlorination degree. In each class the PCB isomers differ from each other only for the position of the chlorine atoms on the aromatic ring (eg 2 of 12 trichlorobiphenyls are the isomers 2,3,4-trichloro biphenyls and 3,3',5-trichloro biphenyls) (Ruzzenenti, 2003). Only 12 of the 209 PCB congeners, the so-called coplanar, have chemical-physical and toxicological properties comparable to dioxins and furans: these are called dioxin-like PCBs and marked with the initials DL-PCBs. Molecular size and conformation of the planar PCB congeners are the most important elements in determining their action mechanism. These structural features are dependent on the number of chlorine atoms and especially on their positions (ortho, meta and para) on the biphenyl molecule. This structural similarity ensures that the coplanar PCBs act, at the cellular level, in a way similar to the 2,3,7,8-TCDD, interaction that is not possible for the non-planar congeners, known as non-dioxin-like PCBs (NDL-PCBs). In fact, DL-PCBs effects on human health and on other organisms are similar to those reported for dioxins.

3.2 Chemical And Physical Aspects

From the chemical and physical point of view PCBs have a high stability, which is also dependent on the different degree of chlorination. In general the evaporation point and lipophilicity increase with the greater degree of chlorination, the vapor pressure and the solubility in water decreases instead. The most important characteristics of PCBs are:

- liquid state at room temperature,
- density: 1.1821566 kg/L,
- low solubility in water, high solubility in organic solvents,
- high evaporation temperature: 170-380 °C,
- non-explosive,
- low electrical conductivity,
- very high thermal conductivity,
- high stability: high resistance to thermal and chemical degradation (Fiedler, 2001).

3.3 PCBs toxicity and release into the environment

Once arrived in to the environment, PCB are not easily destroyed and can persist for long periods of time, due to their characteristic of being strongly bioaccumulative. They can easily move between air, water and soil. The presence of polychlorinated biphenyls in the environment and their diffusion occurs by evaporation or leaching and transport with aerosols or by adsorption on dust particles. In not contaminated regions, the PCBs measured content in the air is 0.003 ng/m^3, in polluted regions up to 3 ng/m^3 (Deml, 2000). From the atmosphere, PCBs through dust deposition and precipitation may reach soil, surface water and plants. Polychlorinated biphenyls may be found in particularly high concentrations also in aquatic organisms and in the soil (Deml, 2000).

Lower molecular weight PCBs can be found in the surface layers of water and be transported by currents or evaporate into the air; heavier PCB are found adhering to the sediments where they remain fixed, or are transferred to the surrounding water. Human exposure to these substances seems to be a consequence of the redistribution that PCBs undergo once introduced into the environment. As the degree of volatilization and degradation of polychlorinated biphenyls depending of the different congeners, they are redistributed in different proportions in the environment (WHO, 1992). Their degradation depends on the biphenyl degree of chlorination. In general, the persistence of the different congeners increases with the number of chlorine atoms. The microorganisms are able to quickly degrade the mono-, di-and trichlorobyphenyls, and rather slowly the tetrachlorobiphenyls. The congeners with a higher degree of chlorination are not biodegraded. Even the different position of the chlorine atom affects the environmental fate of these substances: congeners which have a chlorine atom in the *para* position are anaerobically processed, congeners with a lower degree of chlorination are subjected to an easier aerobic degradation (WHO, 1992). Polychlorinated biphenyls in water are adsorbed to sediments and organic material. The close link with the sediment, especially for congeners with more chlorine atoms, decreases the amount of substance that can evaporate. The congeners with a lower degree of chlorination instead adsorb less permanently. In this way, however, PCBs are immobilized in aquatic sediments for a certain period, even if these substances are with time again released into the aquatic environment, which thus becomes a reserve of PCBs capable of still recycle in the environment.

4. POLYBROMINATED DIPHENYL ETHERS (PBDE)

The polybrominated diphenyl ethers, commonly indicated with the PBDE acronym, can be considered "emerging chemical pollutants," even though the first news of their discovery in aqueous matrix date back to the '80s. With the improvement of the detection limits associated with the instrumental methods used for the determination of these compounds, cases of PBDEs positivity in the aquatic environment have significantly increased in recent years to the point to identify their presence in human consumption water.
Their growing popularity, mostly related to their use as flame retardants, is undergoing a significant arrest, because their role as toxic and potential carcinogen and as endocrine disruptors was confirmed.

4.1 Structure

The basic structure of biphenyl (Figure 4.1) allows the presence of a number of bromine atoms between 1 and 10, with different degree of substitution for the two benzene rings and different arrangements of these atoms within each ring.

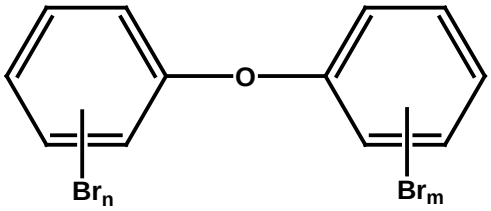

Figure 4.1 Basic structure of bromo biphenyl

Consequently, there are 209 different congeners which, by analogy with what happens for polychlorinated biphenyls (PCBs), are classified according to the IUPAC nomenclature by an identification number, increasing with the number of bromine atoms in the structure. The possibilities that arise are summarized in Table 4.1.

Table 4.1 Chemical aspects of PBDE

FORMULA	NAME	NUMBER OF ISOMERS	IUPAC NUMBER	MOLECULAR WEIGHT	% W/W Br
$C_{12}H_9BrO$	Mono-BDE	3	1-3	248,97	32,09
$C_{12}H_8Br_2O$	Di-BDE	12	4-15	327,87	48,74
$C_{12}H_7Br_3O$	Tri-BDE	24	16-39	406,77	58,93
$C_{12}H_6Br_4O$	Tetra-BDE	42	40-81	485,66	65,81
$C_{12}H_5Br_5O$	Penta-BDE	46	82-127	564,56	70,77
$C_{12}H_4Br_6O$	Esa-BDE	42	128-169	643,45	74,51
$C_{12}H_3Br_7O$	Epta-BDE	24	170-193	722,35	77,43
$C_{12}H_2Br_8O$	Octa-BDE	12	194-205	801,25	79,78
$C_{12}H_1Br_9O$	Nona-BDE	3	206-208	880,14	81,71
$C_{12}Br_{10}O$	Deca-BDE	1	209	959,04	83,32

4.2 Use

PBDEs are mainly used as flame retardants in stuffing pillows and mattresses, but also for containers of televisions and personal computers. Their low flammability and slowness to propagate flames should be such as to allow people to escape in case of fire.
From the commercial point of view, not all 209 congeners are significant: the only three commercial products of note, called DE-71, DE-79 and DE-83r, are actually a mixture of congeners as shown in Table 4.2.

Table 4.2 Characteristics of the main commercial mixtures of PBDEs

Commercial product	Composition	Principal use
DE-71 (pentaBDE)	24-38% tetraBDE 50-60% pentaBDE 4-12% hesaBDE	flame retardant in polyurethane foams for furniture, mattresses and car seats.
DE-79 (octaBDE)	0.1% triBDE 0.5% pentaBDE 12% hesaBDE 45% heptaBDE 33% octaBDE 10% nonaBDE 0,7% decaBDE	flame retardant in ABS plastics (acrylo nitrile-butadiene-styrene) for containers of TV and PC
DE-83 (decaBDE)	0.3-3% nonaBDE 97-99% decaBDE	flame retardant in high impact polystyrene (HIPS) for TV and textiles for coating.

The hazardous PBDE properties in relation to human are gradually leading to the banning of derived commercial products.

4.3 Toxicity

In mammals, PBDEs are mainly absorbed by fatty tissue (adipose tissue, adrenal gland, gastrointestinal tract, skin and liver). The studies so far made on the effects of PBDE against mammals mainly involved mice and other rodents, and the results obtained can be summarized as follows:

- ¾ hepatic effects: there was a significant incidence of cases of liver enlargement, greater in male specimens. With reference to the commercial mixtures, those dipentaBDE and OctaBDE caused more serious damage than those of decaBDE ;
- ¾ immunological effects: rats treated with commercial mixtures of pentaBDE accused the suppression of antibody formation, decreased thymus weight, reduction in the number of lymphocytes and splenocytes;
- ¾ neurobehavioral alterations: phenomena of hyperactivity and various types of behavioral inconsistencies was observed;
- ¾ effects on the endocrine system: endocrine disrupting activity was observed against the system of the thyroid gland, with possible neurological impairments and mental retardation. Inhibition of endogenous androgenic activity were also observed, with the possible development of tumor formation and difficulties in reproduction;
- ¾ reproductive effects: delay in the development of this apparatus, and, in males, decreased sperm production;
- ¾ carcinogenicity: carcinogenicity by decaBDE was found on rats, especially in the liver. There are no known studies on the possible carcinogenicity of pentaBDE and OctaBDE .

5. FOOD REGULATORY FRAMEWORK

5.1 Dioxins, furans and polychlorinated biphenyls

The exposition to PCDD/Fs and DL-PCBs indicate that a considerable part of the European population assumes these substances with food. The regulatory framework in the field of dioxins, furans and polychlorinated biphenyls is constantly evolving. Each document issued by the European Commission calls on the Member States to carry out a monitoring of the presence of these substances in proportion to their production, their use and their consumption of feed and food. Under these controls, if the action limits are exceeded, the Authorities must initiate investigations to identify the source of contamination and to take measures to reduce or eliminate them.

At the end of 2011 Regulation (EU) No 1259/2011, amending Regulation (EC) no. 1881/2006 as regards the maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs, was published on the Official Journal of the European Union no. L 320 of 3.12.2011.

This Regulation introduces substantial changes to the maximum permissible limits defined above, it is totally mandatory and directly applicable in all Member States from 1st January 2012. The maximum limits laid down by Commission Regulation (EU) No 1259/ 2011 for PCDD/Fs and DL-PCBs was lowered for almost all food matrices. In particular, the sum of dioxins (PCDD/F) in milk, milk by-products, eggs and muscle of cattle and sheep, is reduced from 3.0 to 2.5 pg-TEQ/g fat, while the sum of PCDD/Fs and DL-PCBs is changed from 6.0 to 5.5 pg-TEQ/g fat for milk and eggs, and from 4.5 to 4.0 pg-TEQ/g fat for meat. The maximum level of PCDD/Fs in pig muscle and adipose tissue (1.0 pg-TEQ/g fat) is unchanged while the upper limit for the sum of PCDD/Fs and DL-PCBs is reduced from 1.5 to 1.25 pg-TEQ/g fat for both matrices.

The same regulation included for the first time the NDL -PCBs, those PCB that do not have a dioxin similar toxicity. Six NDL-PCBs have been identified as markers or indicators (PCB 28, 52, 101, 138, 153 and 180), their sum comprises about half of the total amount of non dioxin-like PCBs in food and feed. This amount is considered as an appropriate marker for the presence and for the exposure assessment to non-dioxin-like PCBs. Therefore also for these substances limits have been established. The maximum levels of NDL -PCBs were established on the basis of recent data presented in the EFSA scientific report entitled "Results of the monitoring of non-dioxin-like PCBs in food and feed" (Results of surveillance of non dioxin-like PCBs in food and feed).

Regulation 1259/2011 has also introduced, for the first time, the specific maximum levels for dioxins and dioxin-like PCBs in baby food, fixing 0.1-pg TE/g for PCDD/F and 0.2-pg TE/g for the sum of PCDD/Fs and DL- PCBs. Moreover, the EU Regulation established the maximum limits also for food products containing less than 1% fat which so far had been excluded, taking into account the fact that these products usually play a limited role in the exposition of people but that, as recent studies shown, they also contain high levels of these contaminants. The last regulatory update, in March 2012 (Council Regulation (EU) no. 277/2012 of the Commission), regards the maximum and threshold values of dioxins and PCBs in animal feed and the sampling and laboratory analysis requirements. The change of the maximum tolerable levels for dioxins and DL-PCBs and the definition for the first time of maximum levels for NDL-PCBs, as well as the need to update the criteria for screening methods, required a change in the rules governing the determination of dioxins and PCBs in feed contained in Part B of Annex V to Regulation (EC) no. 152/2009.

To this end, in 2012 also Regulation (EU) No. 278/2012, on the amendment of Regulation (EC) no. 152/2009 of the Commission of 27 January 2009, was issued. This regulation defines the methods of sampling and analysis for the official control of animal feed and the methods for the determination of levels of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated (PCDFs) and polychlorinated biphenyls (PCBs), dioxin-like PCBs in feed.

Recently, even the Regulation (EU) No 1259/2011 has been integrated with the Recommendation 711 of 3 December 2013 on the reduction of the presence of dioxins, furans and PCBs in feed and food. This Recommendation modifies the level of action. The action levels are a tool, for competent authorities and operators, to highlight those cases where it is appropriate to identify a source of contamination and to take measures for its reduction or elimination. The recommendation states that, since the sources of dioxins and dioxin-like PCBs are different, action levels should be determine separately.

The impressive legislative efforts on updating the legislation on dioxins and PCBs confirms that the standard of food safety assurance are getting higher and cogent and that the general approach expressed by the concept of "from farm to fork" is confirmed as the most effective solution to the problems that the trade consumption and globalization made increasingly important.

5.2 Polybromobiphenyls

European Community has adopted legislation to reduce or end the sale and use of certain brominated flame retardants (BFRs) including PBDEs, in order to protect human health and environment.
Directive 2003/11/EC, amending Directive 76/769/EEC on admission on the marketing and use of certain dangerous substances and preparations, forbids the sale of two commercial mixtures of PBDEs, known as pentaBDE and OctaBDE, in concentrations higher than 0,1 % w/w. Since July 2006, in accordance with Directive 2002/95/EC, all electrical and electronic equipment can no longer contain PBB and PBDE, in any concentration. In July 2008 also the decaBDE, which had originally been exempted from the restrictions, was banned by the European Court of Justice. In November 2005 the European Commission asked EFSA to identify the chemical compounds belonging to brominated flame retardants that may be of concern to human and/or animal health, in order to monitor their presence in food and feed. On its opinion of February 2006, EFSA shown some compounds belonging to the five major classes mentioned above to be monitored in food and feed, based on the knowledge available at that time on production volumes, the presence of each chemical in food and feed, their persistence in the environment and their toxicity. Subsequently, in October 2006 an EU-wide collection of data on the presence of these compounds in food started. In June 2009, to assess the need for regulatory measures with regard to the presence of brominated flame retardants in food, in accordance with Article 29, paragraph 1 of Regulation (EC) no. 178/2002, the Commission asked EFSA five scientific opinion on the risks to human health related to the presence of BFRs in food (Council Regulation (EC) no.178/2002 on food la-EUR-Lex).
The risk assessment activities and the development of regulatory measures have been carried out by the Scientific Panel on Contaminants in the Food Chain (CONTAM). Between October 2010 and October 2012, the group published scientific advices on monitoring for the presence of PBDEs in food and feed, and assessed the potential risk to human health related to the presence of PBDEs in food.
Eight PBDE were considered of primary interest and toxicity data were available for four of them (BDE -47, -99, -153 and -209). The risk assessment was limited to these four, which were used for the approach of the Margin of Exposure (MOE). For BDE-99, the MOE indicates a potential problem for health with regard to the general dietary exposure. This is relevant for young children (aged 1-3 years), although the presence of a food sample with a high concentration of BDE-99 in the category "Food for infants and small children" could have led to an exposure overestimation for this specific age group. For BDE-47,-153 and -209 is unlikely that the actual exposure through diet may raise health concerns. Since many products containing PBDEs are still in use, the monitoring of PBDEs should not be interrupted (www.europass.parma.it) .

6. AIMS OF THE STUDY

The main objective of the research activity was the development of a method to determine the real contribution of the daily diet to the toxic and persistent contaminants exposure.

This study aims to provide an estimate of exposure to certain pollutants such as dioxins, PCD, and PBDE for people living in different geographical areas of Italy, discerning also for consumer age (toddlers, children and adults) and diets.

Nowadays, people have less time to prepare their foods and they usually eat in canteens, fast food or restaurants, thus our attention was focused on the investigation of food and drinking water of some Italian canteen and restaurant meals. In particular meals were collected to form different composites depending on the source and type of meal.

Several methods can be used to estimate the intake of a chemical substance through foods (WHO 1985. Kroes et al 2002):

- selective studies of individual foodstuffs (SSSF),

- *Duplicate diet* studies (DDS)

- *Total diet* studies(TDS)

- *Market basket* studies (MBS).

Our attention is focused on Total diet studies (TDS) and Duplicate diet studies (DDS), through the analysis of canteens and restaurants meals from Italian adults and children and the analysis of double portions of all meals (24h) prepared at home by some adults respectively.

The analysis of these ready to eat meals, carried out by gas chromatography coupled to mass spectrometry, will allow us to provide a preliminary overview of the actual exposure to chemical contaminants through food.

7. EXPERIMENTAL SECTION

7.1 Sampling

Sampling was carried out through the selection of six different types of meals, depending on the following different dietary habits:
(1) consumption of baby food by toddlers aged 9-12 months (Baby food, BF)
(2) consumption of meals in school canteens for children attending nursery and primary school (age 4-9 years), and canteens for adults (aged 18-64 years).
(3) consumption of fast food meals by adults (Fast Foods, FF)
(4) consumption of food prepared at home by adults (Duplicate diet, DD)
(5) consumption of meals, that exclude meat, fish and poultry, as adults they observe a vegetarian lifestyle (Vegetarian diet, V)
(6) consumption of "Sundays meals " at the restaurant by adults (Restaurant meals, RM).
The canteen meals were collected in collaboration with four Italian primary schools (Ferrara, Perugia, Genoa-1, Genoa-2), a nursery school (Portici, NA) and a canteen that provides meals for office (Brescia) (Figure 7.1).

Figure 7.1 Geographical distribution of sampling on the national territory.

In general, each meal was composed of a main dish consisting of pasta or soup, a second course (meat, fish, eggs or cheese), side dishes and fruit or dessert (Figure 7.2).

Figure 7.2. Example of sampling and homogenization of a complete school meal.

Daily canteen servings over five consecutive days in Brescia, Genoa, Ferrara and Portici, were sampled on site and their contents are listed in the following tables (Table 7.1 and 7.2)

Table 7.1 List of all servings included in the five-days lunch meal composite in Brescia and Ferrara canteens.

Day	Brescia	Weight (g)	Ferrara	Weight (g)
Monday	Risotto	300	Pasta with tomatoes	180
	Turkey	150	Chicken with herbs	75
	Spinaches	120	Organic salad	30
Tuesday	Chocolate pudding	125	Gnocchi	270
	Spicy pasta	320	Halibut	120
	Milanese cutlet	145	Peas	88
	Cauliflower gratin	200		
	Pear yoghurt	125		
Wednesday	Lasagna Bolognese	280	Pasta with zucchini	224
	Breaded baked ham with cheese	150	Parma ham	50
	Pulse salad	200	Baked potatoes	128
	Vanilla pudding	125		
Thursday	Vegetable soup	200	Vegetable cream soup with rice	330
	Hake	140	Turkey	80
	Fresh vegetables	130	Salad	50
	Baked apple	120		
Friday	Gnocchi with Gorgonzola	230	Pasta with meat sauce	210
	Omelette	190	Mozzarella	95
	Potatoes	220	Tomatoes	90
	Fruit salad	200		
	Strudel	120		
All servings		3790		2020

Table 7.2 Meals collected from school canteens of Genoa and Portici

Day	Genoa-1	Weight (g)	Genoa-2	Weight (g)	Portici	Weight (g)
Monday	Vegetable cream soup with pasta	300	Soup	361	Pasta with pumpkins	226
	Cod sticks	100	Fish sticks	128	Fried fish	74
	Salad	77	Salad	64	Green beans	31
	Parmesan cheese	5			Apple	105
	Apple	141				
Tuesday	Pasta with butter	219	Pasta with butter	197	Pasta with potatoes	268
	Pork loin with apples	259	Roast pork loin with apples	143	Omelette	74
	Carrots	75	Carrots	53	Pear juice	223
	Parmesan cheese	6				
Wednesday	Lasagna	209	Lasagna	170	Pasta with beans	342
	Turkey with lemon	171	Chicken with lemon	134	Baked ham	46
	Cabbage salad	137	Cabbage salad	26	Tomatoes	43
					Apple	53
Thursday	Pasta with pesto	292	Pasta with pesto	242	Rice salad	156
	Stracchino cheese	96	Stracchino cheese	101	Fish sticks	76
	Baked potatoes	69	Baked potatoes	137	Zucchini	62
					Pear juice	222
Friday	Pasta with tomato sauce	231	Pasta with tomato sauce	273	Pasta with tomato sauce and ricotta	161
	Cheese	6	Meatloaf	143	Chicken roulade	70
	Meatloaf with green beans	126			Spinaches with butter	81
	Chocolate pudding	97			Apple	96
All servings		2641		2172		2409

Fast food meals (Table 7.3) (pizza, chicken nuggets and meat dishes) were collected at three different fast food restaurants in Rome city centre.

Table 7.3 List of all servings included in the fast food meals composite

Fast food Restaurant	Serving	Weight (g)
Fast food-1	Chicken nuggets	131
	French fries	70.1
	Apple	80.9
Fast food-2	Sandwich	99.6
	Chicken cutlet	115
	Tomatoes	67.4
	Salad	74.1
	Mozzarella	24.8
	Olives	10.8
	Boiled corn	45.7
	Tomatoes	34.6
	Carrots	8.17
	Oil	4.58
	Vinegar	10
	Salt	1.20
	Chocolate cake	124
Fast food-3	Pizza	226
	Arancini (rice balls)	106
	Fruit salad	184
All servings		1419

Duplicate diet consisted in the duplicate portions of all foods consumed during one day by seven persons from Rome and a vegetarian meal served in an office canteen of the capital. The details of the dishes are presented in the following tables (Table 7.4 and 7.5)

Table 7.4 List of all servings included in the duplicate diets composite

Individuals	Meal	Serving	Weight (g)	Individuals	Meal	Serving	Weight (g)
1	**Breakfast**	Bread	30.0	**4**	**Breakfast**	Corn Flakes	30.0
		Jam	10.0			Milk	190
		Butter	5.0		**Lunch**	Pasta with pesto	288
		Milk and coffee	245			Muffin	34.4
		Sugar	10.0		**Dinner**	Mixed vegetable gratin	117
		Coffee	10.0			Potato flan	295
	Lunch	Meatballs	160	**5**	**Breakfast**	Milk and coffee	113
		Baked potatoes	199			Biscuits	68.5
		Cherries			**Lunch**	Pasta with tomato sauce	343
		Dinner				Vegetable soup	250
		Meatloaf			**Dinner**	Omelette	43.7
		Chicken frankfurters				Bread	31.4
	Dinner	Salad	15.0			Apple	137
		Egg	60.0	**6**	**Breakfast**	Milk and coffee	155
		Oil	3.00			Brioche	27.5
		Bread	20.0		**Lunch**	Pasta with tomato sauce	379
		Banana	117			Apple	132
2	**Breakfast**	Corn Flakes	27.3		**Dinner**	Mozzarella	94.1
		Milk	183			Parma ham	64.1
	Lunch	Sandwich	102			Coral beans	195
		Tuna	40.6			Bread	59.1
		Tomatoes	48.2	**7**	**Breakfast**	Biscuits	42.3
		Apple	104		**Lunch**	Spelt salad	332
		Corn biscuits	13.0		**Dinner**	Soft cheese	176
	Dinner	Frankfurter	147			Mixed salad	43.9
		Sauerkraut	183				
3	**Breakfast**	Biscuits	55.8				
		Milk and coffee	204				
	Lunch	Cod	139				
		Vegetables	34.1				
		Chicory	175				
		Yoghurt	125				
	Dinner	Salad with chicken	115				
		Bread	67.4				
		Kiwi	73.9				
All servings							6749

Table 7.5 Vegetarian meal served in an office canteen

Vegetarian meal	Serving	Weight (g)
	Potato and zucchini flan	284
	Boiled peas, carrots, mushrooms	215
	Fruit salad	217
All servings		715.96

The baby food meal was prepared by mixing individual samples of foods for infants (N=44, three most sold brands considered representative of each single food item) to form a composite simulating a five days diet, according to paediatric nutritional recommendations for 9-12 month toddlers (Table 7.6).

Table 7.6 Meal by toddlers aged 9-12 months.

Food item	Weight (g)
Vegetable broth	23.8
Vegetable soup	110
Cereal flours	82.5
Homogenised legumes	16.5
Homogenised meat *veal* *poultry* *rabbit* *ham* *lamb*	55.0
Homogenised fish *hake* *trout* *plaice* *sea bream* *salmon*	27.5
Egg yolk	11.0
Olive oil	16.5
Cheese	11.0
Homogenised fruit	165
All food items	510

The contribution of milk formulas (N=19) to diet has been taken into account separately.
Restaurant meals, selected as more elaborated meals usually consumed "on Sunday" by Italian adult population, were collected in collaboration with secondary hotel-school located in Vico Equense (NA), during the school training activities, over seven non-consecutive days. All samples were delivered to the laboratory where they were weighed and assembled, forming a total of 17 composites. The composition and the total net weight of the meals collected from hotel-school located in Vico Equense and indicated by the letters from RM-A to RM-G (RM, Restaurant meal) are reported in Table 7.7.

Table 7.7 Meals collected from hotel-school located in Vico Equense

Composites	Serving	Weight (g)
RM-A	Lasagna	509
	Loin of Pork	268
	Black sauerkraut	213
	Desserts	231
	All servings	1221
RM-B	Risotto	238
	Grouper Roulade	93.0
	Desserts	371
	All servings	702
RM-C	Lagane with chick peas and shrimp	549
	Escarole roulade	83.0
	Rump steak and cauliflower	198
	Neapolitan pastiera pie	187
	All servings	1017
RM-D	Agnolotti	128
	Braised in Barolo	103
	Mashed potatoes	95.0
	Fresh baked ricotta and pear	179
	All servings	505
RM-E	Tagliatelle Bolognese	263
	Savory pies	353
	Potatoes and carrots	148
	Neapolitan pastiera pie	200
	All servings	964
RM-F	Sartù rice	464
	Sliced beef with tomato sauce	277
	Baked potatoes	249
	Sweet "Paris Brest"	160
	All servings	1150
RM-G	Pimps baked with ham and cheese	277
	Apple pie and chocolate cake	124
	All servings	401

Since PCDD, PCDF, PCB-dl and PBDE, because of their lipophilic nature, manly occur in lipid-richfoods of animal origin, water and water-based beverages were not included in the composites. The vegetarian meal was collected to achieve an approximate background exposure.

7.2 Materials and Methods

The method for the analysis of PCDD/Fs and PCBs gives considerable importance to the cleaning of glassware and to the purity of the reagents used: the glassware can be a source of sample contamination but could also subtract the interest analytes to the analysis through adsorption on the surfaces of the glass.

The interfering compounds (chlorinated biphenyls PCBs, polychlorinated methoxy-, idrofenildifenileteri, benzilfenileteri, polynuclear aromatic and pesticides are the most frequent) can be found at much higher concentrations than those reported for dioxins. Since very low levels of contaminants are measured with this method, the elimination of these interferences is essential. For this reason it was decided to use ultrapure solvents, or at least reagents tested for pesticides, and to pretreat each piece of glassware which come into contact with samples or labeled standards with a solution of sulfuric acid and ammonium persulphate (ammonium Persulfate 35% w/w in sulfuric acid). Subsequently steps of rinsing were performed with tap water,

milliQ water and finally with RS acetone (special reagent). At the end of the treatment the glassware was dried in an oven at 250 °C for 3 hours. The function of ammonium persulfate is to oxidize any residual organic trace with the resulting formation of active sites on the surface of the glass.
The sample, before being subjected to instrumental analysis, undergoes several steps which may be summarized in: sample pretreatment, extraction and purification (Figure 7.3).

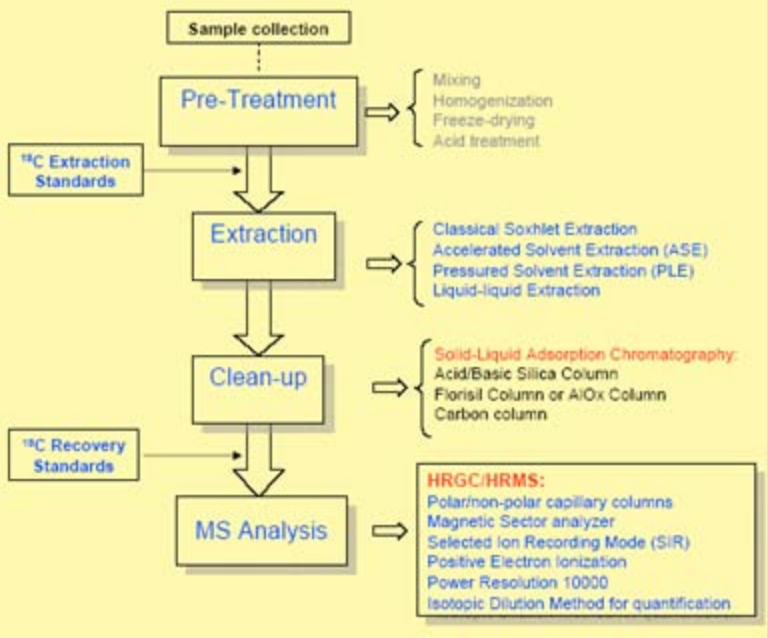

Figure 7.3: Analytic procedure scheme

Samples were frozen at -20°C for 24h, homogenized and divided in aliquots (10g each) to perform the analysis. The lipids naturally present in the sample and co-extracted together with the analytes of interest may interfere with their determination. Therefore the lipid content must be reduced or eliminated by using the purification procedure shown below. The quantitative analysis was performed with the method of isotope dilution by adding, before the extraction process, the corresponding ^{13}C-labeled (internal standard), reported in Table 7.8.

Table 7.8 – Labeled internal standards.

Internal Standard	Concentration ng/mL
$^{13}C_{12}$ 2,3,7,8-TCDD	100
$^{13}C_{12}$ 2,3,7,8-TCDF	100
$^{13}C_{12}$ 1,2,3,7,8-PeCDD	100
$^{13}C_{12}$ 1,2,3,7,8-PeCDF	100
$^{13}C_{12}$ 2,3,4,7,8-PeCDF	100
$^{13}C_{12}$ 1,2,3,4,7,8-HxCDD	100
$^{13}C_{12}$ 1,2,3,6,7,8-HxCDD	100
$^{13}C_{12}$ 1,2,3,4,7,8-HxCDF	100
$^{13}C_{12}$ 1,2,3,6,7,8-HxCDF	100
$^{13}C_{12}$ 1,2,3,7,8,9-HxCDF	100
$^{13}C_{12}$ 2,3,4,6,7,8-HxCDF	100
$^{13}C_{12}$ 1,2,3,4,6,7,8-HpCDD	100
$^{13}C_{12}$ 1,2,3,4,6,7,8-HpCDF	100
$^{13}C_{12}$ 1,2,3,4,7,8,9-HpCDF	100
$^{13}C_{12}$ OCDD	200
^{13}C-T_4BDE	100

^{13}C-P$_5$BDE	100
^{13}C-H$_7$BDE	100
^{13}C-N$_9$BDE	100
^{13}C-D$_{10}$BDE	100
^{13}C-T$_4$BDE	100

Sample is enriched in the phase of instrumental analysis (standard syringe or injection) with the standards shown in Table 7.9.

Table 7.9 - Syringe standard.

Syringe standard	Concentration ng/mL
$^{13}C_{12}$ -1,2,3,4-TCDD	200
$^{13}C_{12}$ -1,2,3,7,8,9-HxCDD	200
^{13}C- Clordano (xPBDE)	200

All standards were purchased by specialized companies (Wellington Laboratories) that provide certificates relating to the congener identity, the degree of purity (99%), and their real title. The working solution of extraction marked standards, DIOX EXTRA, was obtained by dilution of a EDF-8999mother mixture. The concentrations for dioxins and PCBs are shown in Table. 7.10

Table. 7.10 Labeled standards

COMPONENT	EDF-8999 Conc. (ng/mL)	PURITY	DIOX EXTRA Conc. (ng/mL)
2,3,7,8 Tetra-CDD $^{13}C_{12}$	100.00	99 %	0.5
1,2,3,7,8 Penta-CDD $^{13}C_{12}$	100.00	99 %	0.5
1,2,3,4,7,8/ 1,2,3,6,7,8 Esa-CDD $^{13}C_{12}$	200.00	97 %/99 %	1.0
1,2,3,4,6,7,8 Epta-CDD $^{13}C_{12}$	100.00	99 %	0.5
Octa-CDD $^{13}C_{12}$	200.00	98 %	1.0
2,3,7,8 Tetra-CDF $^{13}C_{12}$	100.00	99 %	0.5
1,2,3,7,8 Penta-CDF $^{13}C_{12}$	100.00	99 %	0.5
2,3,4,7,8 Penta-CDF $^{13}C_{12}$	100.00	97 %	0.5
1,2,3,4,7,8 Esa-CDF $^{13}C_{12}$	100.00	99 %	0.5
1,2,3,6,7,8 Esa-CDF $^{13}C_{12}$	100.00	99 %	0.5
1,2,3,7,8,9 Esa-CDF $^{13}C_{12}$	100.00	98 %	0.5
2,3,4,6,7,8 Esa-CDF $^{13}C_{12}$	100.00	98 %	0.5
1,2,3,4,6,7,8 Epta-CDF $^{13}C_{12}$	100.00	99 %	0.5
1,2,3,4,7,8,9 Epta-CDF $^{13}C_{12}$	100.00	97 %	0.5

The labeled compounds have chemical-physical characteristics equivalent to the analytes and have a different mass/charge value, deriving from the stable isotopic structure used.

PCDD, PCDF and DL-PCB were quantified by high-resolution gas chromatography coupled to a high-resolution mass spectrometry (HRGC-HRMS). The selected PBDE congeners (28, 47, 99, 100, 153, 154, 183 and 209), considered of primary interest(EFSA2011a), were determinated by high-resolution gas chromatography coupled to a low-resolution mass spectrometry(HRGC-LRMS). The quality control was assured by analysis of replicate samples (3x); quality assurance was assured by the regular participation of the laboratory to proficiency tests, under accreditation conditions. Results were reported as pg WHO $_{1998/2005}$-TE g^{-1} of PCDD, PCDF and DL-PCBs, and as $ngkg^{-1}$ for PBDE congeners, and expressed on the basis of whole weight (ww), using the following approaches:

- Upper bound (UB): value calculated by assuming the contribution of each non-quantified congener equal to the limit of quantification.
- Lower bound (LB): value calculated by assuming the contribution of each non-quantified congener as zero.

7.3 Procedure

The procedure steps are listed below:

7. 3.1 Liquid-liquid extraction with ASE (Dioxins and PCBs)

The extraction with pressurized fluid has been realized through an ASE 350 extractor (Accelerate Solvent Extraction) by Thermo Scientific Dionex Corp (Figure 7.4).

Figure 7.4 ASE Extractor

This instrument uses n-hexane at elevated temperatures and pressures (100 atm) to increase the efficiency of the extraction process. Into a beaker 10 g of analyte freeze-dried (Christ Alpha 1-4 lyophilizerlsc) and homogenized (200 Grindomix shredder knives, Retsch) and 10g of Diatomaceous Earth Hydromatrix were introduced and mixed using a glass rod. Subsequently the mixture was introduced into the steel cell extractor using a funnel and a piston. After the upload, a spatula of anhydrous sodium sulfate and a glass fiber filter were added.

The cells have subsequently been closed and positioned in the slots of the instrument in correspondence each with a collection bottle glass. The extraction was carried out in n-hexane. The obtained extract was concentrated to small volume through a ROTOVAPOR IKA RV 10.

7.3.2 Soxhlet Extraction (PBDE)

The extraction was carried out for 18-24 hours with n-hexane (about 150 recycle). The extract was concentrated under reduced pressure and weighed to pick up a 5% w/W quantity to be used for the lipid content determination. This was calculated by leaving the aliquot under a slight nitrogen flow until it reaches a constant weight.

7.3.3 Purification

Purification was performed by multilayer column chromatography (De Felip et al., 1990, 1999). The multilayer column was prepared and packaged as follows (from bottom to top): two spoon of anhydrous Na_2SO_4, two of silica gel, 35 g of Extrelut mixed with 40 g of concentrated sulfuric acid, two spoon of silica gel and a spatula of anhydrous Na_2SO_4 (Figure 7.5).

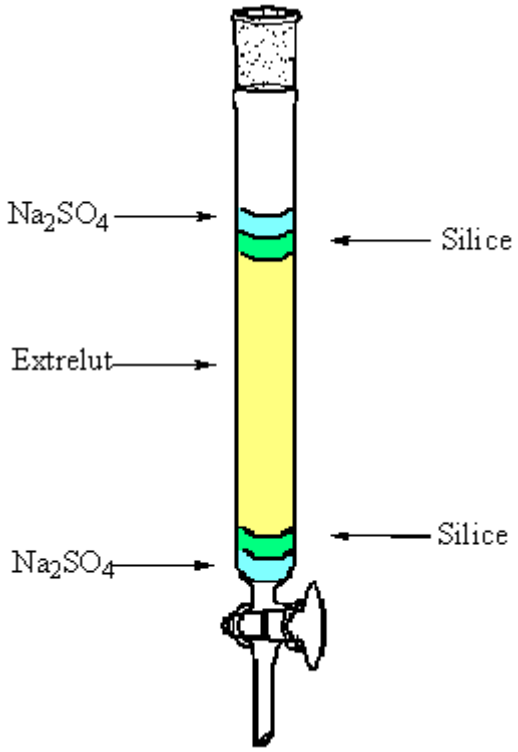

Figure 7.5 Chromatographic column scheme.

The column was packed with hexane and then loaded with the concentrated extract, effecting 3 washes of the sample tube with approx. 0.5-1 mL of n-hexane.
The elution solvents was a 200 mL mixture of toluene/n-hexane 1/9. The purified extract was collected and concentrated to small volume (1 mL) by TurboVap II (Biotage) (T=40 °C, P~0.6 bar).

7. 3.4 Dioxins, PCB and PBDE separation

The eluate from the concentrated acid column was subjected to further purification by Power Prep ™ Fluid Management System (LabService), an automatic purification system constituted by single use columns containing silica multilayer teflon, alumina and activated carbon, for the separation of PCDDs/PCDFs from PCBs and PBDE (Figure 7.6). The instrument is equipped with five solvents or ultrapure solvent mixtures (N-Hexane, Toluene, Ethylacetate/toluene 1/1, Dichloromethane/n-hexane 1/1, Dichloromethane 8% in n-Hexane) loading lines and a drain, two lines of collection for each module, and a system of eight valves. Everything is managed by the "DMS 6000"software.

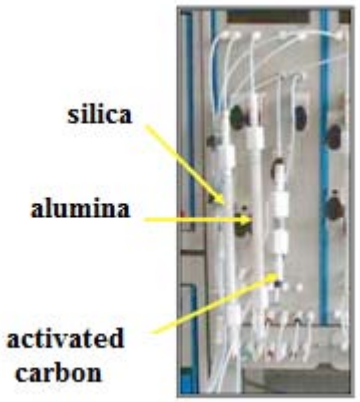

Figure 7.6. Power Prep columns

The multilayer silica column (ABN = acid-base-neutral) is used to eliminate interferents and fats. The alumina column retains PCDD/F, PCB and PBDE which are then differently eluted changing the solvents and their proportions. In particular, the sample is sequentially eluted with 80 mL of n-hexane, 150 mL of a 50:1 mixture of

n-hexane-dichloromethane (Fraction I, containing the NDL-PCBs and mono-ortho-DL-PCBs), 190 mL of a 1:1 mixture of n-hexane-dichloromethane (Fraction II, containing the PBDE), and finally 70 mL of toluene at reverse flow (Fraction III, containing PCDD + PCDF and non-ortho-DL-PCBs).
While the multilayer silica column and the alumina column work for polarity, the graphitic carbon column acts for physical affinity of the molecules according to the flatness. The extract was collected in a tube for the TurboVap (apparatus for concentrating under nitrogen flow) and was concentrated (T = 40°C, P~0.6 bar) to small volume (ca. 1 mL). After adding 1 ml of n-Tetradecane, fractions of interest were concentrated almost to dryness under nitrogen and then resumed by 50-100 µL of iso-octane containing the standard injection shown in Table 7.10.

7.4 HRGC-HRMS analysis of PCDD/F and DL-PCB

Analysis of dioxins and furans was performed by HRGC/HRMS high resolution analyzer with dual fire (magnetic field followed by an electrostatic field) by Thermo Scientific DFS.
The dry sample was dissolved in 10 µL of syringe standard for dioxins and PCB. The MAT 95 XP mass spectrometer is a double focusing instrument with Nier-Johnson inverse geometry (the magnetic sector precedes the electrostatic). The MAT 95 XP system is controlled by a microprocessor (with ICL software, Instrument Control Language) that communicates *via* Ethernet cable with the acquired data system computer (Xcalibur software). All connections between the microprocessor and the operator are carried out *via* a PC using the Xcalibur software.
1 µL of injection volume was used in "sandwich" mode, with a air buffer before and after the sample collection. The auto sampler platform was thermostated at 18 °C. In order to avoid contamination and entrainment, several washes with toluene were carried out before injection, and repeated washings (also 10 for each solvent) after the injection with different polarity solvents (methanol, acetone, n-nonane, toluene).

The gas-chromatographic conditions adopted for PCDD/F are summarized in the following table 7.11:

Table 7.11

Temperature (°C)	*Time (min)*	*Hold(min)*
100	-	2.0
220	12.0	10.0
235	3.0	7.0
315	4.4	2.0

The temperature of the transfer line is at 300 °C, while the injector SSL is at 280 °C.
The gas-chromatographic conditions adopted for DL-PCBs are summarized in the following table 7.12:

Table 7.12

Temperature(°C)	*Time (min)*	*Hold(min)*
90	-	1.0
180	22.5	0.0
285	2.8	0.0
320	11.7	0.0

The temperature of the transfer line is at 300 °C, while the SSL is 280 °C.
For both PCDD/F for DL-PCBs the method provides seven acquisition windows with the masses (m/z) corresponding to the congeners with different chlorination degree. The quantitative analysis was performed using the isotope dilution method by adding before the extraction process, for each congener (except OCDF and 1,2,3,7,8,9-HxCDD) the corresponding ^{13}C-labeled (internal standard). For every analyte and its corresponding labeled compound two molecular ions were monitored.
For a correct identification is necessary to be sure that the two specific ions, selected for each group of congeners, satisfy calculated relative abundance relationships for each analyte in the standard at the lowest concentration used to construct the calibration curve and reported in the instrument software.
Acceptability of these values is directly given from the instrument.

- Quantitative Analysis

The instrument's software analyzes the ratio between the area of each congener and those of the corresponding ^{13}C labeled internal standard added at the beginning of the analysis and directly calculates the concentration in ng/mL of each analytes through the calibration lines.

Results were after converted as pg$WHO_{1998/2005}$TE/g for PCDDs, PCDFs and DL-PCBs, using TEF of each congener, and the WHO-TEQ value is then calculated as the sum of each TEQs.

For each sample, TEQ is calculated using the lower bound (LB) and upper bound (UB) approach: in the first case, TEQ is calculated only by adding the quantified congener concentrations while in the UB case, the value of the concentration of congener corresponds to the LOQ.

-Criteria and Requirements for the approval or rejection of evidence:

For each test, the following specific requirements must be verified:

General specifications: the difference between the upper-bound and lower-bound level must not be higher than 20% for foodstuffs with a contamination of about 1 pg WHO-TEQ/g fat (based on the amount of PCDD/Fs and PCBs). For low in fat food, the same requirements for contamination levels of about 1 pg WHO-TEQ/g product are used. For the lower levels (0.5 pg WHO-TEQ/g product) the difference between the upper-bound and lower-bound level could be between 25% and 40%.

- Control of recoveries:

Recovery rates for the ^{13}C-labeled internal standards were within the 60-120% range. Lower or higher recoveries, in particular for some dibenzo-p-dioxins and some hepta and octachlorinated compounds are acceptable if their contribution to the TEQ value does not exceed 10% of the total TEQ (based on the total PCDD/Fs and PCBs). Instrumental resolution for each sample must be at least 10000 at the 10% of the signal. Instrument sensitivity is considered acceptable when, during the chromatographic run of the lower concentrations standard, the S/N ratio of 2,3,7,8-TCDD compared to the background, is greater than or equal to 10.

7.5. HRGC-LRMS analysis of PBDE

PBDEs are determined by HRGC-LRMS Trace GC Ultra, Thermo Electron Corporation (EI, 35 eV) in SIM mode (single ion monitoring) using the operating conditions described below:

- column gas chromatography: HT-5 (5% phenyl (equiv) carborane-polysiloxane, 25 m × 0:22 Øi mm × 0.1 uM) or equivalent;
- carrier gas: He;
- PTV injector or on-column;
- GC program: 80 ° C for 1 min, 30 °C/min up to 240 ° C, 10 ° C / min up to 340 °C, 340 °C for 4 min;

The PBDE congeners relevant to the investigations are reported in the following table.

Table 7.13 PBDE Congeners

CONGENERS	NO. CAS
2,4,4′-T_3BDE [28]	
2,2′,4,4′-T_4BDE [47]	5436-43-1
2,2′,3,4,4′-P_5BDE [85]	182346-21-0
2,2′,4,4′,5-P_5BDE [99]	60348-60-9
2,2′,4,4′,6-P_5BDE [100]	189084-64-8
2,2′,4,4′,5,5′-H_6BDE [153]	68631-49-2
2,2′,4,4′,5,6′-H_6BDE [154]	207122-15-4
2,2′,3,4,4′,5′,6′-H_7BDE [183]	
2,2′,3,3′,4,4′,6,6′-O_8BDE [197]	
2,2′,3,4,4′,5,5′,6-N_9BDE [206]	
D_{10}BDE [209]	1163-19-5

8. RESULTS AND DISCUSSION

8.1 PCDD/F, PCB and PBDE dietary exposure calculation.

In order to assess dietary exposure of a population to contaminants, occurrence and food consumption data were used. The average amount of solid food consumption derived from the national food consumption dataset (INRAN, 2005) was set as 44,1 g kg^{-1}bw day^{-1} for 4-9 years old children and 17,7 g kg^{-1}bw $day^{-1}$for 18-64 years old adults.

Dietary exposure (E exposure) was calculated as the product of the cumulative TE levels of PCDD, PCDF, DL-PCBs, and analytical levels of PBDE in each composite (C) for the consumption of solid food (Q):

$$E = C \times Q$$

C for dioxins and PCB: pg WHO_{2005}-TE g^{-1}ww
C for PBDE: ng g^{-1}
Q for PBDE: ng kg^{-1}Body Weight Day^{-1}
Q for dioxins and PCB: pg-TE kg^{-1}Body Weight Day^{-1}

As regards Italian toddlers (9-12 months old), the dietary exposure was estimated adding up the contributions coming from baby food and from milk formulas. The average daily food consumption (normally consisting of two main meals) derived from the weight of the baby food composite (BF). The occurrence data for milk formulas marketed in Italy (N= 19) were recovered from the scientific literature (Ceci et al. 2012). The default value of 8,8 bw for European 6-12 months aged infants proposed by EFSA (EFSA 2012a) was adopted.

Cumulative intakes were expressed for PCDD, PCDF, DL-PCB in pg-TE kg^{-1}bw day^{-1} using both WHO_{1998} and WHO_{2005}scales (LB and UB) to allow a direct comparison of these estimates with those from previous studies.

PBDE intake estimates were expressed in ng kg^{-1} bw day^{-1}(LB and UB) for each of the eight congeners and their sum. Since health guidance based values for PBDE congeners were not yet established, the margin of exposure (Margin of Exposure, MOE) approach was used for the risk assessment (EFSA 2011).

The MOE was calculated comparing the human intake associated with the body burden at the Benchmark Dose (BMD) essential to estimate the intake of contaminants by diet and to compare them with the relevant guidance values, such as the tolerable daily intake (TDI) or the tolerable weekly intake (TWI).

The Margin of Exposure (MOE) is a tool used by risk assessors to characterize the risk of exposure to carcinogenic and/or genotoxic substances in food. It is necessary to calculate the MOE for those substances for which a toxic threshold able to induce a detrimental effect on health have not been defined and for those substances for which the limited information available does not allow the determination of *Tolerable Daily Intake* (TDI).In general, the MOE is calculated using the following equation:

$$MOE = \frac{NOAEL}{EHE}$$

NOAEL (No Observed Adverse Effect Level)
EHE (Estimated Human Exposure)

MOE is therefore the ratio of two factors with which it is possible to calculate for a population the dose at which an adverse effect is not observable, but still measurable, and the level of exposure to the considered substance. Higher is the MOE, lower is the potential health risk(EFSA 2011 a).

8.2 Concentrations of PCDD, PCDF, DL-PCB and PBDE in prepared meal composites.

The cumulative WHO_{2005}-TE and TE-WHO_{1998} obtained for PCDDs, PCDFs and DL-PCBs in the analyzed composites are reported in Table 8.1, while Table 8.2 reports the analytical values for the eight PBDE congeners considered.

Table 8.1 Cumulative PCDD+PCDF+DL-PCB $WHO_{1998/2005}$-TEcontaminations ($pgWHO_{2005}$-$TEg^{-1}ww$) measured in meal composites from canteens, fast food (FF), baby food (BF), duplicate diets (DD), vegetarian diet (V),and restaurant (RM).

	PCDDs+PCDFs+DL-PCBs			
Composites	**pg WHO_{2005}-TE g^{-1}ww (LB-UB)**		**pg WHO_{1998}-TE g^{-1}ww (LB-UB)**	
Toddler meals BF	0.004	0.02	0.01	0.02
Children meals Ferrara	0.01	0.02	0.01	0.02
Perugia	0.005	0.02	0.01	0.02
Portici	0.001	0.01	0.001	0.01
Genoa-1	0.004	0.02	0.01	0.02
Genoa-2	0.004	0.02	0.005	0.02
Adult meals Brescia	0.002	0.02	0.004	0.01
FF	0.0002	0.02	0.001	0.02
DD	0.01	0.02	0.01	0.02
V	0.01	0.02	0.01	0.02
RM-A	0.007	0.02	0.01	0.02
RM-B	0.01	0.02	0.02	0.03
RM-C	0.01	0.02	0.01	0.02
RM-D	0.03	0.04	0.03	0.04
RM-E	0.02	0.03	0.02	0.03
RM-F	0.01	0.02	0.02	0.03
RM-G	0.01	0.02	0.01	0.02

Table 8.2 PBDE analytical contamination ($ng\ kg^{-1}$) measured in meal composites from canteens, fast food (FF), baby food (BF), duplicate diets (DD), vegetarian diet (V), and restaurant (RM).

Composites	**Tri BDE 28**	**Tetra BDE 47**	**Penta BDE 99**	**Penta BDE 100**	**Hexa BDE 153**	**Hexa BDE 154**	**Hepta BDE 183**	**Deca BDE 209**	**Sum PBDEs (LB-UB)**
Toddler meals									
BF	<0.72	2.02	<1.80	<1.80	<2.89	<2.89	<3.61	<72.2	2.02-15.7
Children meals									
Ferrara	<0.60	3.53	<1.49	<1.49	<2.40	<2.40	<2.98	<74.7	3.53-14.9
Perugia	<0.55	2.78	<1.41	<1.37	<2.21	<2.21	<2.75	<68.8	2.78-13.3
Portici	<0.61	1.93	<1.53	<1.53	<2.45	<2.45	<3.06	<76.6	1.93-13.6
Genova-1	<0.69	2.91	<1.65	<1.65	<2.64	<2.64	<3.31	<82.6	2.91-15.5
Genova-2	<0.79	<1.72	<1.72	<1.72	<2.74	<2.74	<5.72	<150	0.00-17.2
Adult meals									
Brescia	<0.65	<1.51	<1.51	<1.51	<2.41	<2.41	<3.02	<75.5	0.00-13.0
FF	<0.75	<1.90	<1.88	<1.88	<3.01	<3.01	<3.76	<94	0.00-16.2
DD	<1.02	3.85	2.43	<1.55	<2.47	<2.47	<5.60	<74.3	6.28-19.4
V	<0.61	1.68	<1.53	<1.53	<2.46	<2.46	<3.07	<61.2	1.68-13.3
RM-A	<1.11	2.94	2.36	<2.32	<3.48	<3.48	<5.82	<157	5.29-21.5
RM-B	<0.59	4.36	3.63	<2.73	<4.11	<4.11	<6.84	<166	7.98-26.3
RM-C	<0.73	3.84	2.45	<1.79	<2.87	<2.87	<3.59	<89.7	6.29-18.2
RM-D	<0.90	4.72	5.41	<2.25	<3.60	<3.60	<4.51	<90.0	10.13-25.0
RM-E	<0.83	5.12	5.66	<1.95	<3.12	<3.12	<4.21	<85.8	10.78-24.4
RM-F	<0.70	3.71	1.87	<1.75	<2.80	<2.80	<3.50	<87.4	5.58-17.1
RM-G	<0.82	2.25	<2.34	<2.05	<3.27	<3.27	<4.10	<102	2.25-18.1

The PCDDs, PCDFs and DL-PCBs cumulative TE-values (UB) observed ranged between 0.01 (Portici canteen composite) and 0.04 (restaurant meal RM-D) pg WHO_{2005}-TE_{g}^{-1}ww (Table 8.1). Differences between the UB/LB estimates did not exceed 20%, thus respecting with Regulation 252/2012/EU (EC 2012). As to PBDEs, almost all the selected congeners levels were below the limit of quantification (LOQ) except for BDE-47 and BDE-99 (Table 8.2). In children meals, the highest BDE-47 concentration was found in Ferrara canteen (3.53 ng kg^{-1}ww) while, restaurant meal RM-E showed the highest values: 5.12 ng kg^{-1}ww for BDE-47 and 5.66 ng kg^{-1} ww for BDE-99. Lowest PBDE concentrations were found in the vegetarian meal (V) (Table 8.2).
The pooling of different servings from the same location in one composite basically hampers the possibility to trace back the source of the found differences in the contamination between the composites considered. Since foods of animal origin generally shows higher levels of bioaccumulating pollutants than that of vegetable origin (EFSA 2011a, 2012b EFSA),the levels observed in restaurant meals RM-D and RM-E (Table 8.2) were probably caused by the additional presence, in the above menu, of meat based dressing re-fill in the first courses (i.e.tagliatelle alla bolognese and agnolotti) and of dessert prepared with milk and dairy products (Table 7.6). Such explanation is supported from the lowest PBDE level in the vegetarian meal (V) (Table 8.2).

8.3 Estimated dietary intake

Table 8.3 and 8.4 show the estimates of dietary intake of PCDDs, PCDFs, DL-PCBs and PBDEs in toddlers (BF composite), children (from Ferrara, Perugia, Portici, Genoa-1 and Genoa-2 composites) and adults (from Brescia canteen composite, FF composite, DD composites, V vegetarian meal and RM restaurant meal).

Table 8.3 Exposure to PCDDs + PCDFs + DL-PCBs (pg $WHO_{1998/2005}$-TE kg^{-1}bw day^{-1}) produced by different composites.

	PCDDs+PCDFs+DL-PCBs			
Composites	**pg WHO_{2005}-TE kg^{-1}bw day^{-1}**		**pg WHO_{1998}-TE kg^{-1}bw day^{-1}**	
	LB	**UB**	**LB**	**UB**
Toddlers BF[a]	nr[b]	0.67	nr	nr
Children Ferrara	0.44	0.92	0.53	0.93
Perugia	0.22	0.69	0.27	0.66
Portici	0.03	0.63	0.03	0.57
Genoa-1	0.19	0.72	0.23	0.68
Genoa-2	0.18	0.80	0.22	0.75
Adults Brescia	0.04	0.27	0.06	0.26
FF	0.004	0.38	0.01	0.29
DD	0.12	0.32	0.14	0.31
V	0.14	0.33	0.18	0.34
RM-A	0.12	0.34	0.15	0.33
RM -B	0.24	0.44	0.29	0.45
RM -C	0.21	0.38	0.25	0.37
RM -D	0.47	0.63	0.54	0.64
RM -E	0.38	0.57	0.44	0.58
RM -F	0.23	0.44	0.29	0.45
RM -G	0.17	0.42	0.21	0.41

[a]only values in pg WHO_{2005}-TE kg^{-1}bw day^{-1} (UB) including milk formula contribution
[b] nr= not reported

Table 8.4. Estimated exposure of the eight PBDE congeners (ng kg^{-1}bw day^{-1}, UB) and their sum (ng kg^{-1}bw day^{-1}, LB-UB) obtained by the different composite

Composites	Tri BDE 28	Tetra BDE 47	Penta BDE 99	Penta BDE 100	Hexa BDE 153	Hexa BDE 154	Hepta BDE 183	Deca BDE 209	Sum PBDEs (LB-UB)
Toddlers									
BF [a]	0.15	2.75	1.26	0.60	0.71	0.71	0.75	16.43	23.36
Children									
Ferrara	0.03	0.16	0.07	0.07	0.11	0.11	0.13	3.29	0.16-3.95
Perugia	0.02	0.12	0.06	0.06	0.10	0.10	0.12	3.03	0.12-3.62
Portici	0.03	0.09	0.07	0.07	0.11	0.11	0.13	3.38	0.09-3.98
Genoa-1	0.03	0.13	0.07	0.07	0.12	0.12	0.15	3.64	0.13-4.32
Genoa-2	0.03	0.08	0.08	0.08	0.12	0.12	0.25	6.62	0.00-7.38
Adults									
Brescia	0.01	0.03	0.03	0.03	0.04	0.04	0.05	1.34	0.00-1.57
FF	0.01	0.07	0.04	0.03	0.05	0.05	0.06	1.59	0.00-1.91
DD	1.37	0.07	0.04	0.03	0.04	0.04	0.10	1.32	0.11-1.66
V	0.02	0.03	0.03	0.03	0.04	0.04	0.05	1.08	0.03-1.32
RM-A	0.02	0.05	0.04	0.04	0.06	0.06	0.10	2.78	0.09-3.16
RM-B	0.01	0.08	0.06	0.05	0.07	0.07	0.12	2.93	0.14-3.40
RM-C	0.01	0.07	0.04	0.03	0.05	0.05	0.06	1.59	0.11-1.91
RM-D	0.02	0.08	0.10	0.04	0.06	0.06	0.08	1.59	0.18-2.04
RM-E	0.01	0.09	0.10	0.03	0.06	0.06	0.07	1.52	0.19-1.94
RM-F	0.01	0.07	0.03	0.03	0.05	0.05	0.06	1.55	0.10-1.85
RM-G	0.01	0.04	0.04	0.04	0.06	0.06	0.07	1.81	0.08-2.13
[a]only values in pg WHO_{2005}-TE kg^{-1}bw day^{-1} (UB)including milk formula contribution.									

Toddlers and children are expected be more susceptible than adults because of higher food consumption relatively to their body weight (EFSA 2012A). The estimates of this study are in line with such assumption except those obtained for dioxins and DL-PCBs from restaurant meals RM-D and RM-E, whose values 0,63 and 0,57 pg WHO_{2005}-TE kg^{-1}bw day^{-1} are near close to the values reported for the toddlers (0.67 pg WHO_{2005}-TE kg^{-1}bw day^{-1}) and for children of the nursery school of Portici (0.63 pg WHO_{2005}-TE kg^{-11}bw day^{-1}) (Table8.3). These higher intakes reflect the presence of animal fat in the meals and of dessert prepared with milk.

For PBDEs, the discussion on dietary exposure is only focused on the congeners BDE-47 and BDE-99, whose concentrations are above the LOQ (Table 8.2), in agreement with the evidences from other studies (Domingo, 2012).

8.4 Data analysis

Analyzing prepared meals rather than raw food may represent a more realistic way to assess the dietary exposure to contaminants, since they reflect the actual contamination of the food as really consumed, taking into account the possible variations of the concentration due to the cooking and contribution of contact materials and cookware (Hori et al 2005; Schecter et al 1996).

Several European studies already investigated dietary exposure to PCDDs, PCDFs and DL-PCBs (Table 8.5), fewer those investigating PBDEs(Table 8.6).

Table 8.5 Overview of the European average dietary intake of PCDDs, PCDFs and DL-PCBs

Country	Year	Kind of study	Population group	Exposure estimates	Units	Estimation	References
Austria	2005–2011	SSSF	Children (6–15 y) Women (19–65 y) Men (19–65 y)	0.50-0.77 0.50-0.75 0.40-0.61	pg WHO_{1998}-TE kg^{-1}bw day^{-1}	LB[a]-MB[b]	Rauscher-Gabernig et al. 2013
Belgium	2008	TDS	Adults (above 15 y)	0.61	pg WHO_{2005}-TE kg^{-1}bw day^{-1}	MB	Windal et al. 2010
Finland	1999	MBS	Adults (25–64 y)	1.58	pg WHO_{1998}-TE kg^{-1}bw day^{-1}	UB	Kiviranta et al. 2004
France	2006–2007	TDS	Children/teenager (3–17 y) Adults (18–79 y)	0.88 0.57	pg WHO_{1998}-TE kg^{-1}bw day^{-1}	MB	Sirot et al. 2012
France	2001–2004	SSSF	Children (3–14 y) Adults (above 15 y)	2.8 1.8	pg WHO_{1998}-TE kg^{-1}bw day^{-1}	LB	Tard et al. 2007
Italy	1994–1996	SSIF	Children nb[c] (0–6 y) Children (7–12 y) Adults (13–94 y)	5.34 3.37 2.28	pg WHO_{1998}-TE kg^{-1}bw day^{-1}	UB	Fattore et al. 2006
Spain	2006–2008	SSSF	Children Adults	4.58 2.86	pg WHO_{1998}-TE kg^{-1}bw day^{-1}	UB	Marin et al. 2011
Sweden	1998–2004	MBS, SSSF	Children 1–3 y (m[d]-f[e]) Children 4–6 y (m-f) Children 7–10 y (m-f)	4.2-4.3 4.5-3.6 2.8-2.7	pg WHO_{1998}-TE kg^{-1}bw day^{-1}	MB	Bergkvist et al. 2008
United Kingdom	2001	TDS	Children (4–18 y) Adults (19–64 y)	0.7-1.8 0.9	pg WHO_{1998}-TE kg^{-1}bw day^{-1}	UB	FSA 2003
The Netherlands	1998-1999	SSIF	infants nb (5 months)	1.1	pg WHO_{1998}-TE kg^{-1}bw	LB	Weijs et al. 2006

[a] LB= lower bound
[b] MB= medium bound
[c] nb= non-breastfed
[d] m= male
[e] f= female

Table 8.6 Overview of the European average dietary intake of PBDEs

Country	Year	Population group	Kind of study	Total PBDEs intake	Units	Estimation	Congeners determined	References
Belgium	2005	General population	MBS	23-35-48	ng day^{-1}	LB-MB-UB	28-47-99-100-153-154-183	**Voorspoels et al. 2007**
Finland	1997–1999	Adults (25–64 y)	MBS	43	ng day^{-1}	LB-UB	47-99-100-153-154	**Kiviranta et al. 2004**
Germany	2005	Adults: m[a]-f[b] (14–60 y)	DDS	1.47-1.20	ng kg^{-1}bw day^{-1}	nr[c]	47-99-100-153-154-183	**Fromme et al. 2009**
Ireland	2006–2007	Adults (18–65 y)	Probabilistic modelling	0.6	ng kg^{-1}bw day^{-1}	ROS	28-47-49-99-100-153-154-183-209	**Trudel et al. 2011**
Norway	2002–2006	Adults (21–80 y)	SSIF	1.4-1.5	ng kg^{-1}bw day^{-1}	LB-UB	47-99-100-153-154	**Knutsen et al. 2008**
Romania	2007	Adults Toddlers (6–24 months)	MBS	40 24	ng day^{-1}	MB	28-47-99-100-153-154-183-209	**Dirtu and Covaci 2010**
Spain	2006	Adult men	MBS	1.1	ng kg^{-1}bw day^{-1}	MB	47-99-100-153-154-183	**Domingo et al. 2008**
Sweden	2005	General population	MBS	31.7-50.6-70.3	ng day^{-1}	LB-MB-UB	28/31-47-66-99-100-138-153-154-183.	**Törnkvist et al. 2011**
The Netherlands	2004	Adults (18–74 y)	DDS	BDE-47: 0.54 BDE-99: 0.41 BDE-209: 0.26	ng day^{-1}	nr	47-99-209	**Zeilmaker et al. 2008**

[a] **m= male**
[b] **f= female**
[c] **nr= not reported**
[d] **ROS= regression on order statistics**

The differences in the methodologies adopted, the food groups considered, the population group studied, the congeners analyzed and expression of results, are reflected in the range of the estimates.
Moreover, dietary intake of contaminants may vary with the consumption habits (Van Audenhaege et al. 2009), such as consuming main meals at home or outside, or such as avoiding specific food items in the diet (eg, vegetarianism, low-fat diets). This can affect the presence/absence of food items particularly contaminated in the individual diet. Only a limited number of studies investigated the dietary exposure to PCDDs, PCDFs, DL-PCBs and PBDEs focusing on the contribution of prepared meals: canteen meals were analyzed in southern Germany to assess the PCDD and PCDF intake (Mayer 2001), fast food meals in Belgium for PBDE exposure of the general population (Voorspoels et al., 2007), infant formulas in a German study for the assessment of dietary exposure to PCDD/Fs and dioxin-like PCBs in children aged 0-9 months (Pandelova et al. 2010), duplicate portions of children with different consumption habits to assess the intake of PCDD/Fs in urban and rural areas Germany (Wittsiepe et al. 2001), vegan and omnivorous duplicate diet samples to assess the UK dietary exposure to PBDEs (Harrad et al. 2004). A recent Korean study measured levels of PCBs in homemade baby food in order to assess the dietary intake of infant aged 6-15 months (Jeong et al., 2013).
This study aims to cover the gap about dietary intake estimates of the persistent organic pollutants in the Italian population. However, the reported results need of course to be interpreted since this study may obviously present some sources of uncertainties depending on both consumption and contamination data.

Food consumption data included solid food only, considering water a negligible contribution to total exposure. Moreover, the single-person consumption behavior has not been taken into account, since average consumption data from a national survey (INRAN 2005) were used for calculations, thus not covering the potential frequent consumption of higher contaminated products.
These estimates were based on the assumption about the whole weekly diet representativeness of the analyzed composites. In this regard, canteens meals represent about 40% of the total food weekly ingested by children attending school and by adults at work (Dellatte et al. 2013). Since the supply chain of the food recognizes a seasonal variation, the different food items chosen in the menu may vary during the year. This can lead to variation in pollutants levels based on the different food items used during the sampling period. The seasonality may therefore represent an uncertainty since this study covered a limited period of time (1-7 days). Regional variations within different menus were partially taken into account, as the meals were collected in the North (Brescia, Genoa-1, 2-Genoa, Ferrara), Central (Perugia, Rome) and the Southern (Portici, Vico Equense) Italy.
The sampling representativeness of the baby food diet formed by 44 baby food and 19 milk formulas can be assumed adequate, considering the high quality standardization of such large scale products, starting from the selection of the raw food suppliers, ending to the adopted food process. The UB estimate is conservative, as far the aggregation of more contaminated with less contaminated food items in composites may determine diluted concentrations falling below the analytical LOQs.

Toddlers
For PCDDs, PCDFs and DL-PCBs the available (UB) cumulative estimates resulted by EFSA for the period 2008-2010 on Italian toddlers indicated 3-fold higher average intakes (2.34 *vs* 0.67pg WHO_{2005}-TE kg^{-1}bw day^{-1})(EFSA2012b) (Table 8.3).
This difference in estimations could be ascribed to the heterogeneity in the EFSA occurrence database, due to the different ranges of sensitivity (LOQ) of the analytical methods, when the UB approach is followed (EFSA 2012b). PCDDs, PCDFs and DL-PCBs intake estimates referred to Italian toddlers consuming baby foods are also lower with respect to data reported for Dutch (1.1 to 2.3 pg WHO1998-TE kg^{-1}bw day^{-1}) (Weijs et al. 2006) and Swedish toddlers (4.2 to 4.3 pg WHO1998-TE kg^{-1}bw day^{-1}) (Bergkvist et al. 2008) (Table 8.5). Such higher dietary exposure could probably be related to the different time frame conduction of the studies (see Figure 8.1).

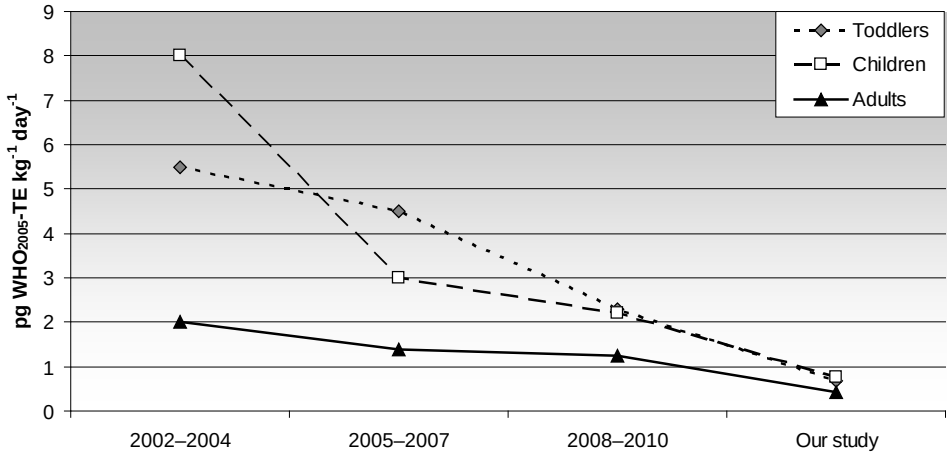

Figure 8.1. Time trends exposure to dioxins and DL-PCBs in children and adults European (comparison between MB and UB estimates, EFSA)

For PBDEs, our results seem to be about six times lower than those reported in the EFSA Opinion on PBDE in food for Italian infants below 1 year old (18.1 and 6.93 vs. 2.75 and 1.26 ngkg^{-1}bw day^{-1}for BDE-47 and BDE-99, respectively) (EFSA 2011a) (Table 8.4.). Even in this regard the difference noted in UB estimates could be ascribed to the different range of sensitivity of the analytical methods.

Children

As for children aged between 4 and 9 years old, the PCDD, PCDF and DL-PCBs UB median cumulative estimates reported by EFSA on Italian children in the period 2008-2010 indicate 3 fold higher intakes (2.15 vs. 0.63-0.92 pg WHO_{2005}-kg^{-1}bw day^{-1}) (EFSA 2012b) (Table 8.3). Lower estimates may reflect the general decreasing tend of the average exposure to PCDDs, PCDFs and DL-PCBs when compared with previous estimates from Italy (3.37 pg WHO_{1998}-TE kg^{-1}bw day^{-1}) (Fattore et al. 2006).

Such decreasing trend in the intakes have been reported in very recent studies from France (0,89 pg WHO_{1998}-TE kg^{-1}bw day^{-1}) (Sirot et al. 2012), Austria (0,77 pg WHO_{1998}-TE kg^{-1}bw day^{-1}) (Rauscher-Gabernig et al. 2013), and United Kingdom (0,95-1,27 pg WHO_{2005}-TE kg^{-1}bw day^{-1}) (Mortimer et al. 2013) (Table 8.5).

Dietary exposure estimates to BDE-47 and BDE-99 (0.08-0.16 and 0.06 to 0.08 $ngkg^{-1}$bw day^{-1}, respectively) (Table 8.6) resulted one order of magnitude lower than those reported by EFSA for European children of 3-6 year old (UB median dietary intake:1.78 and 1.12 and $ngkg^{-1}$bw day^{-1} respectively), and for European children 6-10 year old (1.27 and 0.78 $ngkg^{-1}$ bw day^{-1}).The differences are probably the result of an overestimation of the mean concentration values due to the presence of a unique sample showing an occurrence 40 times higher than the average value (EFSA 2011a). In fact, EFSA occurrence data often result from monitoring programs, with a targeted sampling design, with a consequent possible overestimation of the exposure (EFSA 2012b): the special foods groups or problematic areas, thus capturing highly contaminated food items, may generate occurrence data that do not always reflect the actual whole diet (EFSA 2011b).

Adults

As for infants and children, previous PCDDs, PCDFs and DL-PCBs UB cumulative on Italian adults (EFSA 2012b; Fattore et al, 2006) indicate 2-8 fold higher intakes (1.21 pg WHO_{2005} - TE kg^{-1}bw $day^{-1}$and 2.28 pg WHO_{1998} -TE kg^{-1}bw day^{-1}) than those from this study (0.27 to 0.63 pg WHO_{2005} -TE kg^{-1}bw day^{-1}) (Table 8.3).

This can be explained accounting for the time-trendsand the different sampling strategies adopted, as above discussed for children. Again, the proposed Italian estimates based on prepared meals are in line with the results of recent TDS studies carried out other EU countries, such as Belgium (0.61 pg WHO_{2005}-TE kg^{-1}bw day^{-1}) (Windal et al., 2010), France (0.57 pg WHO1998-TE kg-1 body weight per day) (Sirot et al., 2012), and the United Kingdom (0.52 pg WHO_{2005}-TE kg^{-1}bw day^{-1}) (Mortimer et al., 2013) (Table 8.5), which include food items representative of the whole diet in the sampling plan (EFSA 2011b).

Adults' dietary exposure estimates to BDE-47 and BDE-99 obtained (0.03-0.09 and 0.03-0.10 $ngkg^{-1}$ bw day^{-1}respectively) (Table 8.4) resulted on order of magnitude lower than those reported by EFSA (UB average of 1.2 and 0.43 $ngkg^{-1}$bw day^{-1}) (EFSA 2011a), and in good agreement with those French (EFSA 2011a) (0.092 and 0.042 $ngkg^{-1}$bw day^{-1}).

Other studies on adult from Ireland (0.25 and 0.15 $ngkg^{-1}$bw day^{-1}) (Trudel et al. 2011), the Netherlands (0.54 and 0.41 $ngkg^{-1}$bw day^{-1}) (Zeilmaker et al., 2008) from the United Kingdom (0.20 and 0.14 $ngkg^{-1}$bw day^{-1}) (Mortimer et al., 2013) and Norway (0.69 and 0.16 $ngkg^{-1}$bw day^{-1}) (Knutsen et al.2008) may reflect differences both in investigation methodologies, and in dietary habits and in contamination levels, on geographical basis.

Risk characterization

Assuming that the prepared meals are representative of the whole week diet, all the intake for the Italian different age groups appears to be below the guidance value, the TWI (total weekly intake) of 14 pg WHO_{2005} - TE kg^{-1}bw day^{-1}, set by the Scientific Committee on Food of the European Commission for PCDD/Fs and DL-PCBs in food (EC SCF 2001).

The contribution to the TWI spans from a minimum of 14% (Brescia canteen meal) to a maximum of 32% for adults (restaurant meals RM- D) and for children from a minimum 32% (Portici canteen meal) to a maximum of 46% (Ferrara canteen meal).

This data, when framed within the time trends of the intakes (Figure 8.1) indicates the efficacy of the risk management measures in place within the European Union, on feed and food (EP EC 2002 EC 2006a, 2006b) .

As shown in Table 8.6, for BDE-47, the MOE for all considered age groups were larger than 50: these values might indicate that there is no health concern (EFSA 2011a).

Table 8.6 Margins of exposure (MOEs) for BDE-47 and BDE-99 for adults and children, based on the upper bound (UB) dietary intake ($ngkg^{-1}bw\ day^{-1}$).

Exposed population	MOE	
	BDE-47	BDE-99
Toddlers		
BF	62	3
Children		
Ferrara	1104	64
Perugia	1402	67
Portici	2019	62
Genoa-1	1341	58
Genoa-2	2266	55
Adults		
Brescia	6437	157
FF	2528	97
DD	2525	98
V	5792	156
RM-A	3309	101
RM-B	2231	65
RM-C	2528	97
RM-D	2057	44
RM-E	1897	42
RM-F	2618	127
RM-G	4313	102

However, for toddlers, the computed MOE of 3 for BDE-99 is nearly close to EFSA reported average value of 1.4 (EFSA 2011a), thus confirming the susceptibility of this age group. This MOE value of 3 should be evaluated within a risk-oriented approach, for a possible additional relevant intake through ingestion of dust, due to toddlers' mouthing behavior (Moya et al.2004;Stapleton et al. 2012).

9. CONCLUSIONS

Due to the complexity and expensiveness of conducting long-term studies ,such as TDS, the analysis of composites as result of pooled meals may represent a first cost-effective screening useful to assess persistent organic pollutants intake through food as actually consumed.

Analysis of the data obtained revealed no exposure beyond the legal limits for the population examined , the estimates were lower even than those of the rest of the European population.

In order to refine the dietary exposure assessment it may be useful to perform such screening on regular basis thus reducing possible uncertainties related to the meals representativeness also on seasonal basis.

Such screening approach may allow to obtain an initial approximate estimate in order to decide if further oriented risk assessment supported by implemented monitoring are necessary, as in the case of BDE-99 in toddlers.

10. AKNOWLEDGEMENTS

I would like to gratefully and sincerely thank Dr. Gianfranco Brambilla from the Istituto Superiore di Sanità (ISS), Dr Federica Corrado and Dr Mauro Esposito from the Istituto Zooprofilattico Sperimentale (IZS) del Mezzogiorno.

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